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Day to Day Variability of Ethanol Elimination Rates in Persons Charged With Impaired Driving in Alberta, Canada

by

Bruce David Miller

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

Department of Pharmacology

Edmonton. Alberta

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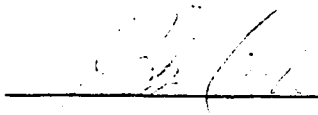

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October 5, 1995

"The great tragedy of Science -
the slaying of a beautiful hypothesis
by an ugly fact."

T. H. Huxley 1825 - 1895
Biogenesis and Abiogenesis

University of Alberta

Faculty of Graduate Studies and Research

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled **Day to Day Variability of Ethanol Elimination Rates in Persons Charged With Impaired Driving in Alberta, Canada** submitted by **Bruce D. Miller** in partial fulfillment of the requirements for the degree of Masters of Science.

William F. Dryden, BSc, PhD

Fakhreddin Jamali, PharmD, MSc, PhD

Glen B. Baker, BSP, MSc, PhD

This Thesis is Dedicated to
My Wife Dawn,
Our Three Children Erin, Megan and Andrew,
And to the Memories of
Jack Taylor, Ph.D.
and
Rhoderick Reiffenstein, Ph.D.

ABSTRACT

Confusion and controversy exist concerning inter- and intra-subject variability of human ethanol elimination rates. Such divergence of opinion appears due to the lack of a scientifically acceptable protocol for measuring elimination rates, coupled with a fundamental misunderstanding of basic pharmacokinetic principles. A computerized, multiple linear regression analyses protocol is introduced providing an objective technique for distinguishing between the α and β phases. The technique was used to measure the elimination rates in over 700 Albertans who had been charged with drinking and driving offences by the police. The average elimination rate for males was 13.5 ± 2.5 mg/ 210 L of breath per hour; for females 15.2 ± 3.26 mg/ 210 L/hour. The intra-subject variability for 29 of the subjects averaged 0.05 ± 1.8 mg/210 L/hour. The correlation of reproducibility for the elimination rates obtained on the first and second occasions was 0.7122.

Acknowledgement

I cannot conceive of this thesis having been possible, let alone completed, without the encouragement and unfailing support of a great number of people.

It has been a very long journey from that initial decision back in 1969 to leave Ottawa and begin university; made all the sweeter by having met a woman of uncommon beauty who would become my friend, my bride, confidant, biggest fan, mother of our three children, and personal editor. Thank you Dawn for all your love, encouragement, support and your expertise in English which helped me make this technical thesis readable.

It is truly amazing the motivational power that the looks of pride and love, emanating from the innocent eyes of our children can have in propelling us over the highest of hurdles. Erin, Megan and Andrew provided such a power, and I thank them for their patience while their daddy descended, yet again, into the "depths" of the basement to spend even more time away from them to write his thesis. I have truly been blessed with three tremendous children.

I have enjoyed the honour of two very unique mentors on my thesis committee. In 1969, Dr. Jack Taylor pioneered the use of Breathalyzers® in Alberta by training the Edmonton City Police to use them. In testament to the standards he espoused, it has only been since his death that those standards have begun to be eroded by the relentless powers of mediocrity. The other great scientific researcher was Dr. Rod Reiffenstein who was one of my first pharmacology professors in the early seventies. Until his recent death, he acted as the chairman of my thesis committee. He was patient, thoughtful, delighted in story telling, and was always there when I needed him. He is sorely missed. Unfortunately, the death of these two great Albertans made membership on my thesis committee somewhat of a risk, and I thank Dr. Bill Dryden for having the courage to take over the responsibility of my thesis committee shortly after Dr. Reiffenstein's death. Dr. Dryden's clarity of thought has proven invaluable.

I would also like to acknowledge the support of Dr. Justin Elbrink, Dr. Glen Baker and Dr. Fakhreddin (Mo) Jamali, who also acted as members of my thesis committee. They never seemed to be distracted by this rather unconventional graduate student.

Finally, I would like to acknowledge the pioneering spirit of all those listed in the bibliography of this thesis who provided the groundwork upon which this thesis could be written. I pray that I

can contribute as much as they to our knowledge of the pharmacokinetics of alcohol.

To all of these people, and too many more to list, I thank you all.

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Symbol	Definition
ACI	Alcohol Concentration Index (BrAC/Normalized Oral Dose)
$ß_{60}$	Hourly Rate of Alcohol Elimination (mg%/hour OR mg/210 L/hour)
BAC	Blood Alcohol Concentration
BrAC	Breath Alcohol Concentration
C_0	Extrapolated Alcohol Concentration at Time Zero
C of V	Coefficient of Variation (%)
DE	Diffusion Equilibrium
D.F.	Degrees of Freedom
F Ratio	Ratio of Sum of Squares Between Groups and Sum of Squares Within Groups for Analysis of Variance
k_0	Zero Order Rate of Decline
mg%	Milligrams of Alcohol per 100 Millilitres of Blood
mg/210 L	Milligrams of Alcohol per 210 Litres of Breath
MS	Mean Sum of Squares for Analysis of Variance
P	Probability of an Occurrence
PI	Ponderal Index (Height/Weight)
SS	Sum of Squares for Analysis of Variance
SD	Standard Deviation
t	Student's t-test for Equality of Means
r^2	Pearson Coefficient of Determination
r	Pearson Correlation Coefficient
r_{PB}	Point Biserial Correlation Coefficient

1. Variability of Ethanol Elimination Rates in Humans

Introduction

In spite of ethanol* having been around since the dawn of man, it has only been in the last hundred years that there has been any attempt to describe in scientific terms the kinetics of this mind altering drug. In 1932, E.M.P. Widmark wrote his now very famous Die theoretischen Grundlagen and die praktische Verwendbarkeit der gerichlich-medizinischen Alkoholbestimmung[1]. Included in his thesis was a very careful study of the rate at which the human body eliminates ethanol. He described two periods in any blood ethanol concentration profile. The first period he described as the absorption period which included not only the uptake of the ethanol into the body, but also the time required to attain "diffusion equilibrium". The subsequent, second period he described as a gradual decrease of the ethanol due to its excretion and metabolism. He measured the rate of this gradual decrease in both men and women, found it to be a constant change per minute, and termed it ß.

Widmark found that there did not seem to be a difference in the rate that women and men eliminate alcohol. In fact, he showed that for men the value for ß was 0.0025 grams per minute with a deviation of ± 0.00056 ($ß_{60}$ = 15 mg% per hour ± 3). For women the mean value of ß was 0.0026 ± 0.00037 ($ß_{60}$ = 16 ± 2). In terms of day to day variability, the difference between the elimination rate for one male subject was less than 1 mg% per hour[2].

In the sixty years since the publication of Widmark's study, much controversy has remained over the actual kinetics of ethanol elimination, and the factors affecting those kinetics. Unfortunately much of the controversy has been caused by the publication of articles describing a vast spectrum of ethanol elimination rates written by those who apparently have not understood the principles of ethanol kinetics, nor the appropriate scientific means for establishing them[3].

Such controversy has been fueled, at least in part, by the forensic application of such knowledge. There exists in Alberta at present a concerted effort by the Crown to prove that individual ethanol

* The term alcohol and ethanol are used interchangeably in this thesis

elimination rates vary so markedly from day to day (by as much as 100%) that they cannot be relied upon by the courts to establish what an accused's blood ethanol concentration, or Breathalyzer® reading should have been based solely upon claimed consumption and the timing of that consumption. Such a position appears to rely on the conclusions of Nagoshi and Willson[4]. In fact, on page 167 of that paper, the authors show one subject having a β_{60} of -22.79 at one time, and a subsequent β_{60} of only -4.80. What is immediately noticeable from their data is that their measurements of β_{60} are not consistent with the normally accepted range of ethanol elimination rates. Unfortunately, their paper fails to identify the period of the ethanol concentration profile during which they were attempting to measuring their β_{60}'s, nor do they provide any indication of the correlation coefficients obtained for their linear regression analyses of the changing breath ethanol concentrations by which they measured β_{60}.

Much mischief can be caused to the course of justice by such publications which neglect basic pharmacokinetic principles in formulating pharmacokinetic conclusions. What is clearly needed is an unambiguous and rigorous investigation of the variability of ethanol elimination rates in a population of human subjects, using accepted pharmacokinetic principles.

Ethanol Pharmacokinetics

The literature on the pharmacology of alcohol is massive. Surprisingly, despite its voluminous nature, there is little that is noncontroversial. While addressing the North American Conference on Ethanol and Highway Safety in 1985, Dr. Frank A. Haight [5] identified two characteristics which have tended to bring into question the value of some of the research on ethanol. In his address, he referred to a report to the U.S. Department of Transportation, prepared by Jones and Joscelyn, which reviewed the protocol and conclusions of approximately 300 current publications. What they noted was that a large body of the research has produced "...ambiguous and vague results.". The second characteristic deals with the motivation for the research. Haight concluded that "We are led to suspect that research on the drinking-driving problem is often motivated less by a desire to discover truths than by a need to abate periods of public turbulence.". In other words, ethanol is a political drug. Because of this one must proceed to a review of the literature with extreme caution.

Gréhant - 1881

The first attempts to describe ethanol elimination kinetics was probably made by Gréhant[6] as early as 1881. During this and subsequent experiments, he injected ethanol intravenously into dogs and found that a considerable period of time was required for the dogs to eliminate the ethanol from the blood.

Michaelis and Menten - 1913

Although Michaelis and Menten[7] made no direct contribution to the ethanol literature of the day, their theory of enzyme saturation kinetics has become critical to our current understanding of ethanol elimination kinetics.

According to this theory, the substrate reacts reversibly with the enzyme to form an enzyme substrate complex. This complex would then break down in a subsequent step, resulting in the product and free enzyme.

$$E + S \underset{k_2}{\overset{k_1}{\rightleftharpoons}} ES \underset{k_4}{\overset{k_3}{\rightleftharpoons}} P + E \quad (1)$$

These reactions are considered to be reversible. The specific rate constants for each of the reactions are designated as k_1, k_2, k_3 and k_4. By this it can be seen that the concentration of free enzyme at any given time would be equal to the total enzyme concentration minus the concentration of bound enzyme, or *[E - ES]*. The rate that the substrate is bound to the free enzyme can be determined by

$$\frac{d[ES]}{dt} = k_1([E] - [ES])[S] \qquad (2)$$

Similarly, the freeing up of the enzyme from the substrate can be given as

$$\frac{-d[ES]}{dt} = k_2[ES] + k_3[ES] \qquad (3)$$

Therefore, when there is an equilibrium between the formation of the enzyme substrate complex, and its breakdown, that is to say a steady state wherein the *ES* complex remains constant, then

$$K_1([E] - [ES])[S] = K_2[ES] + K_3[ES] \qquad (4)$$

This can then be rearranged to give

$$\frac{[S]([E] - [ES])}{[ES]} = \frac{k_2 + k_3}{k_1} = K_M \qquad (5)$$

where K_M is the Michaelis-Menten constant.

Formula (5) can then be rearranged to solve for the steady state concentration of the *ES* complex.

$$[ES] = \frac{[S][E]}{K_M + [S]} \qquad (6)$$

For an enzymatic reaction the initial rate v is proportional to the concentration of the *ES* complex, therefore

$$V = k_3[ES] \qquad (7)$$

As the process continues, if the concentration of the substrate is sufficient to saturate the enzyme, then essentially *[ES]* is maximal

and *[E] - [ES]* is near zero and *[ES]* becomes equal to *[E]*. Under these conditions the velocity is at its maximum and

$$V_{max} = k_3[ES] \qquad (8)$$

A comparison of the initial velocity to the maximum velocity reveals

$$\frac{v}{V_{max}} = \frac{k_3 \frac{[E][S]}{K_M + [S]}}{k_3[E]} \qquad (9)$$

Solving for *v*, the submaximal velocity, results in the Michaelis-Menten equation which provides for the velocity or rate of an enzymatic process when V_{max} and K_M are known.

$$v = \frac{V_{max}[S]}{K_M + [S]} \qquad (10)$$

The value of K_M can be determined when the velocity is half of the maximal velocity (*i.e.* $v = \frac{1}{2} V_{max}$).

$$\frac{V_{max}}{2} = \frac{V_{max}[S]}{K_M + [S]}$$

$$K_M = [S] \qquad (11)$$

On the basis of this simple equation, the value of V_{max} is difficult to establish graphically since its value is approached asymptotically (Figure 1.).

Gabbe - 1917

The first to describe the elimination of ethanol as declining at a constant rate was probably Gabbe in 1917. Newman and Cutting[8] describe how Gabbe repeated Gréhant's experiments but intravenously infused a smaller dose of ethanol into dogs. Following the infusion, he waited 30 minutes for the ethanol to be equilibrated between the blood and the tissues. It was during the subsequent elimination phase that Gabbe noted the change to be constant over time.

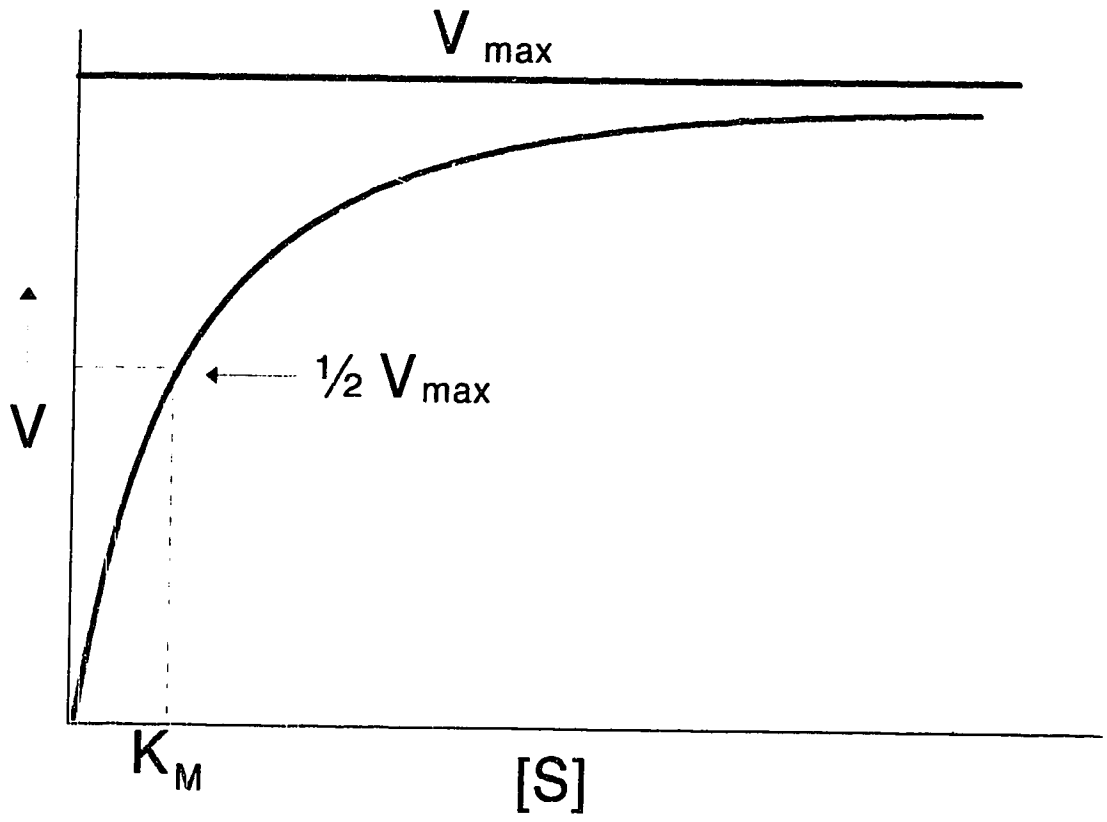


Figure 1. Overall velocity (v) versus the substrate concentration (S), where K_M and V_{max} are respectively the Michaelis-Menten constant and the maximum velocity.

Mellanby - 1919

In his now very famous experiments on dogs, Mellanby attempted to determine the factors which affected the rate of elimination in dogs. Rather than infuse the ethanol intravenously, he administered the alcohol orally[9]. Like Gréhant, he also noted that considerable time was required for the animal to eliminate the ethanol from its body. In one experiment it took a 13.5 kilogram dog 20 hours to eliminate 50 millilitres of ethanol (0.185 ml/kg/hour). Mellanby noted that "...the rate of oxidation is constant throughout the whole period, and this is the case in spite of the fact that the amount of ethanol in the body is getting progressively less."[10]. To show that this linear decline is independent of the amount consumed, Mellanby fed one dog four different doses of ethanol and observed that the declining (elimination) phases of the four resulting blood ethanol curves were remarkably parallel.

Mellanby went on to determine whether exercise alters the rate of ethanol elimination[11]. He described the earlier work of Atwater and Benedict who concluded that ethanol can replace fats and carbohydrates isodynamically in both the resting and active state. Mellanby therefore reasoned that if this was true, then the metabolic rate of ethanol should be greater in the active than in the resting state and must be directly proportional to the overall increase in metabolism due to the increased activity.

During the resting phase of his experiments, Mellanby kept the dogs confined to small cages for the duration of the experiment, about six hours. During the active experiments, the same animals were allowed to roam at large, and were encouraged to keep active. Mellanby observed that when the dogs were given a dose of 4.1 ml/kg there did not appear to be any difference between the elimination rates of the resting and the active dogs. This then appeared to conflict with Atwater and Benedict's isodynamic theory. However, when the dose of ethanol was reduced to 2.5 ml/kg, the dogs eliminated ethanol faster while active than while at rest. This difference was made even more significant when the dose was reduced to only 2.0 ml/kg.

Mellanby reasoned that while activity at lower doses increases the elimination rate, this effect is decreased with increasing blood ethanol concentrations. Mellanby interpreted this to mean that the cellular effect of alcohol is to inhibit complete oxidation of not only itself but of other energy sources as well. The result is an accumulation of metabolic products associated with incomplete

oxidation such as lactic acid, leading to fatigue which is quite apart from the centrally induced fatigue produced by ethanol.

In summation, Mellanby concluded that while at low doses, activity increases the rate of ethanol elimination; at higher doses the ethanol acts to self-limit its oxidation as well as that of other energy sources. This results in fatigue which is associated with a return to elimination rates consistent with non-activity.

Widmark - 1932

Widmark[12] was probably the first to attempt to describe ethanol kinetics in mathematical terms. He used male medical students, aged 19 to 40 years, as subjects for his experiments. In his monograph, he emphasized the need to identify both phases of the curve before making any conclusions about elimination rates.

Following complete alcoholic abstinence for at least 24 hours, each subject was given a measured amount of diluted spirits, *i.e.* diluted ethanol, cognac or brandy to consume on an empty stomach. Before consumption was allowed to occur, blood samples were drawn and analyzed to ensure that residual ethanol concentrations were not outside of the normal concentration. Following consumption further samples were collected at regular intervals. These samples were analyzed using the Widmark dessication method. A slight modification of that method is still currently used by the Royal Canadian Mounted Police Forensic Laboratories. The results were then plotted on a graph to provide the blood ethanol concentration versus sampling time profile.

Absorption Distribution Phase

Widmark was very particular in describing the initial absorption phase of the ethanol concentration curve and distinguishing it from the subsequent post absorptive phase. He explained that this initial phase results from two opposing forces, the input of ethanol from the gastrointestinal tract into the blood, and its distribution into the tissues. This being a relatively short period of time, elimination does not play a dominant role.

Under the conditions of his testing, absorption of the ethanol was generally complete within 60 minutes; no ethanol remained unabsorbed after 110 minutes. He noted that the rate of absorption varied within the same individual as well as between the subjects. It

is important to note that Widmark's absorption period can extend beyond any peak blood ethanol concentration to that part of the curve which shows a declining concentration. The first phase ends when an equilibrium is established between the ethanol concentration in the tissues and the blood. He referred to this as diffusion equilibrium.

The absorption phase does not end abruptly with the establishment of diffusion equilibrium: the end occurs much more subtly. Because of this it is often difficult to accurately determine when the initial phase of the curve ends and the post absorptive phase begins. Diffusion equilibrium is a function of the difference in the concentration of ethanol in the tissues and that in the blood. This is markedly different from the elimination phase of the curve which Widmark showed to be independent of ethanol concentration. He therefore assumed that the end of the initial phase occurred when the ethanol concentration curve is transformed into a straight line. It is this straight line that Widmark argued can be used to measure β, the rate of ethanol elimination.

Elimination Phase

Widmark referred to the elimination of ethanol as "the conversion of ethanol", or the processes through which the ethanol disappears from the body. This therefore included not only its metabolism, but its excretion as well. He was emphatic that ß could only be measured once an equilibrium between blood and tissue ethanol had been established, a point often ignored even in the current literature[13]. He argued that during the initial absorption phase there is no proportionality between the amount of ethanol in the blood and the tissues, that too much variability exists in the initial phase to measure ß.

He conducted a number of experiments to determine characteristics of the elimination rate. He experimented with multiple doses of ethanol to see if the concentration of ethanol in the blood affects elimination rates, and concluded that the rate is linear and independent of the concurrent blood ethanol concentration. He tested to see if the rate is reproducible for the same subject from day to day and found that for the one male subject that he tested, there was no significant difference from the first day ($ß_{60}$ = 14 mg% per hour) to the second day ($ß_{60}$ = 15 mg% per hour). Similarly he found that there was not a significant difference between the mean

elimination rate for males (16 ± 3.5) and for females (16 ± 2.3)[14]. He determined the range of elimination rates for both males and females to be 11 to 25 mg% per hour[15].

He also compared the ethanol content of the blood to the total amount of ethanol in the subject's body. He found the ratio to be constant and termed it *r*. Since ethanol is distributed in the watery tissues of the body, he reasoned that the *r* factor is a function of the concentration of water in the body. A change in the *r* factor did not change ß. Widmark determined that for males the *r* factor = 0.68 ± 0.085 and for females the *r* factor = 0.55 ± 0.055[16]. He reasoned that the difference between the male and female values of *r* is due to the difference in the distribution of fatty tissue between the two genders. In general the *r* factor values decrease with increasing corpulence.

In Summary

Widmark was emphatic that the elimination rate of ethanol ($ß_{60}$) could not successfully be measured prior to the establishment of an equilibrium between the concentration of ethanol in the blood and that in the surrounding tissues. From his testing he concluded that $ß_{60}$ is both linear and a constant, independent of the dose of ethanol, the concurrent blood ethanol concentration or gender of the individual. He also showed that when a male and a female of equal stature consume the same dose of ethanol, the female will generate a blood ethanol concentration about 1/6th higher than her male counterpart. Widmark reasoned that this is due to the differences in the distribution of fatty tissue between the male and female physiques.

Haggard and Greenberg - 1934

Haggard and Greenberg[17] were probably the first to show that the rate of ethanol elimination in dogs is proportional to the concurrent blood ethanol concentration. They took exception to Mellanby's claim that ethanol is eliminated at a constant rate. They argued that the difference between their results and those of Mellanby was that Mellanby had erroneously assumed that absorption was complete at the time that the blood ethanol concentration had reached its maximum, and that any subsequent change was a measure of the rate of elimination.

To guard against any influence that absorption might have on their results, Haggard and Greenberg diluted the ethanol in saline and administered ethanol to their four dogs intravenously. The dose of ethanol ranged from one to three grams of ethanol per killogram body weight (Table 1). In a previous paper[18], they had shown that when ethanol is uniformly distributed throughout the body, both the femeral venous and arterial blood ethanol concentrations become equal. This became their criterion for diffusion equilibrium arguing that "Only after the attainment of this state of equilibrium of distribution is change in the concentration of ethanol in the arterial blood a true criterion of change in the amount of ethanol in the body."[19]. They found that within fifteen minutes following the injection of the ethanol, distribution was complete.

Contrary to the previous findings of Gabbe, Mellanby and Widmark, Haggard and Greenberg observed that the rate of ethanol elimination was not linear, but proportional to the concentration of ethanol in the blood. While the percent rate of decline remained fairly uniform for each of the four dogs, the rate did change from one animal to the other (Table 1, Figure 2.) with a mean rate of 17.6 per cent per hour.

Newman and Cutting - 1935

In 1933 Newman and Mehrtens[20,21] conducted human experiments on the relationship between the ethanol concentration in blood and the corresponding cerebrospinal fluid. Newman observed that following the intravenous injection of between 0.5 and 1.5 millilitres of ethanol per kilogram body weight, the ethanol concentration decreased at a fairly linear rate. He also determined that the blood ethanol concentration could be maintained simply by intravenously infusing ethanol at an uniform rate.

Based upon these observations, Newman and Cutting[22] reasoned that if they could determine the amount of ethanol required to maintain a constant blood ethanol concentration in their human subjects, that this would therefore reflect the rate of ethanol elimination. The protocol followed (Figure 3.) was to initially administer a dose of ethanol followed by a period of time for diffusion equilibrium to occur. The blood ethanol concentration was then determined. This blood ethanol concentration was then maintained by infusing ethanol over time at a uniform rate. The amount of ethanol infused over the time period was then calculated

Table 1.

Dose vs. Hourly Decrease in Blood Alcohol Concentration In Each of Four Dogs Tested

DOG	INJECTION TIME (Minutes)	DOSE (ml/kg)	HOURLY DECREASE (%/Hour) RANGE	AVERAGE
I	90	6	14.1 - 19.2	17.1
II	45	2.5	13.5 - 26.4	21.1
III	60	2.8	11.2 - 21.5	16.4
IV	90	5.5	14.4 - 17.8	15.9

Adapted from Haggard and Greenberg (1934) pages 170 - 172

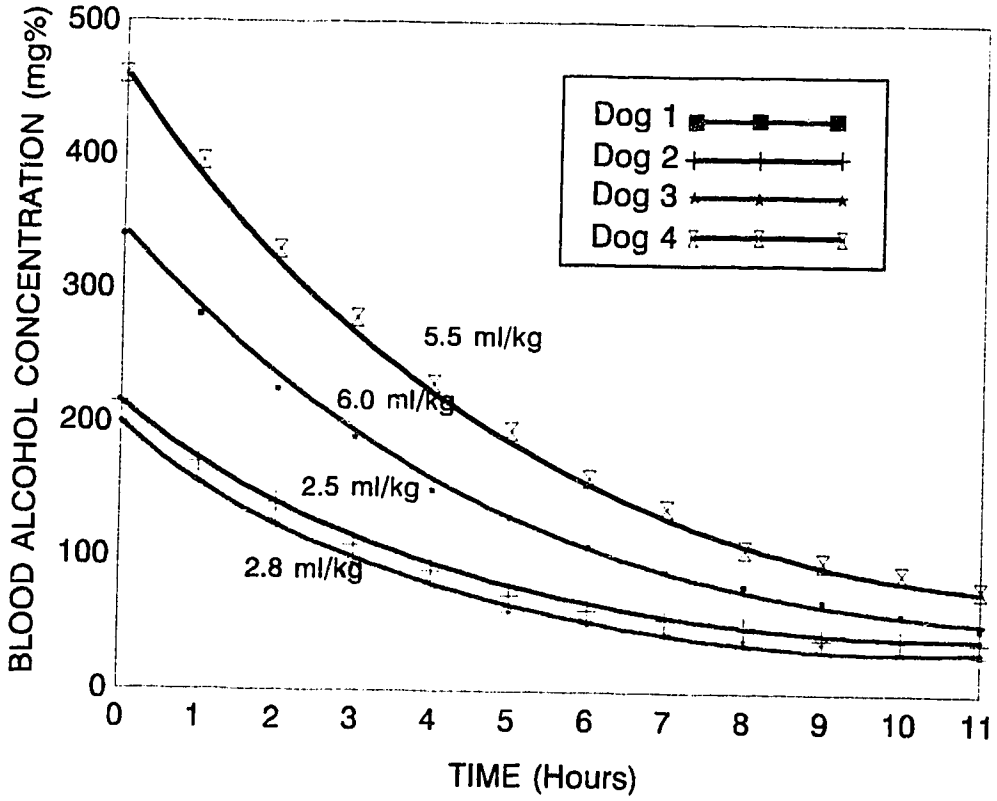


Figure 2. The non-linear decline in the arterial blood alcohol concentration for each of four dogs following intravenous alcohol infusion. The average hourly rate of decline for each of the four dogs was -17.1, -21.1, -16.4, and -15.9% per hour respectively. For the four dogs the average was -17.6% per hour.

Data adapted from Haggard and Greenberg (1934) page 171

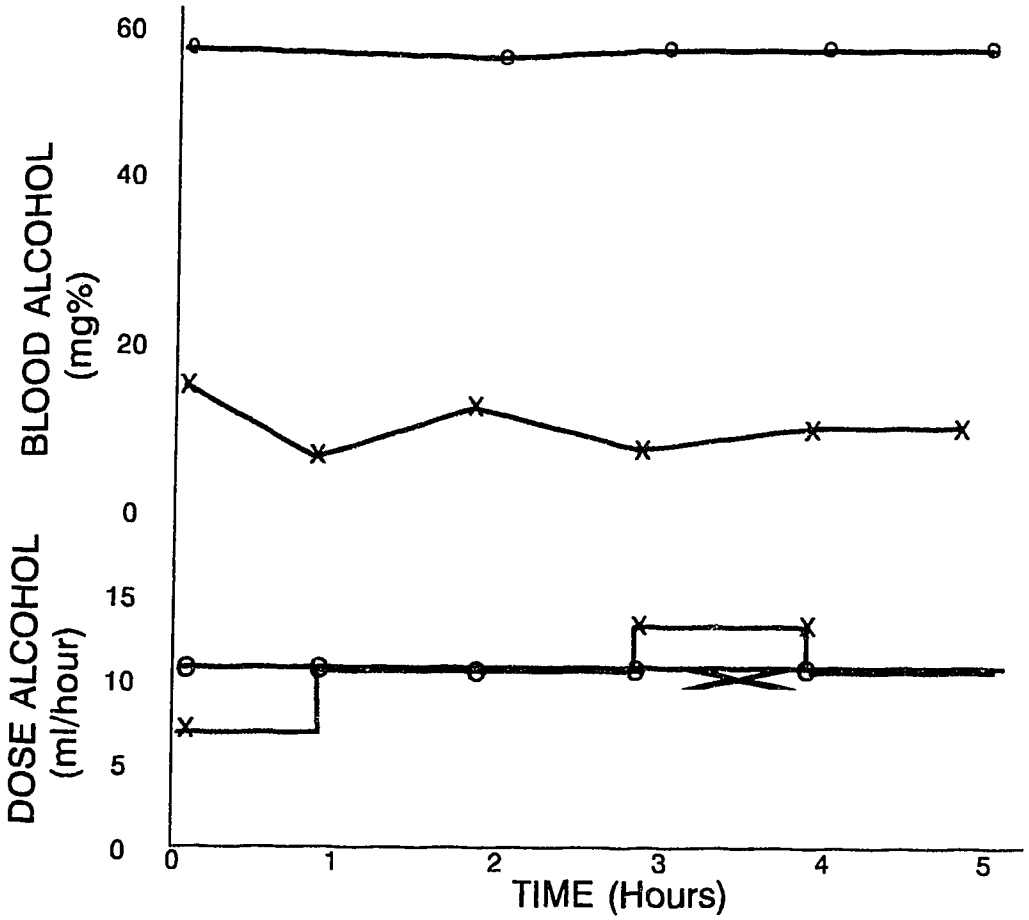


Figure 3. Newman and Cutting maintained in each of two subjects 2 different blood ethanol concentrations on two separate days by infusing ethanol intravenously at a uniform rate. This figure shows that for one subject the blood ethanol concentration on day 1 was 15 mg% (the red "x") forty minutes after the initial injection of 0.33 ml ethanol per kg body weight. Using a similar protocol 3 days later, a blood ethanol concentration of 56 mg% was obtained sixty minutes after the initial injection of 1.0 ml/kg (the blue "o"). On each occasion, these initial blood ethanol concentrations were maintained over a period of five hours. During those five hours, 55 ml of ethanol was administered on the first day, and 56.25 ml on the second day. This corresponds therefore to an infusion rate of 0.167 millilitres of ethanol per kilogram per hour (ml/kg/hour) and 0.170 ml/kg/hour respectively. For the second subject the infusion rate was 0.179 ml/kg/hour for each of the two days tested. The average elimination rate for their two subjects was 0.174 ml/kg/hour. This is not significantly different from the 0.185 ml/kg/hour obtained by Mellanby in his experiments with dogs.

Adapted from Newman and Cutting (1935)

to be the individual's elimination rate.

Consistent with both Mellanby and Widmark, Cutting and Newman concluded that with blood ethanol concentrations less than 100 mg%, the elimination of ethanol in man proceeds at a constant rate regardless of the concurrent blood ethanol concentration. As well they showed that whereas elimination rates may vary from one individual to another, an individual's rate of elimination remains fairly constant from one day to the next, consistent with Widmark's findings.

Newman, Lehman & Cutting - 1937

Prior to 1937, Newman and Cutting had concluded that the rate of ethanol elimination is independent of the concurrent blood ethanol concentration. However, in collaboration with Lehman, they showed that the ethanol elimination rate is affected by the dose of the ethanol administered.[23] They intravenously infused a dose of between 1 and 6 ml ethanol per kg to each of ten dogs. After allowing for equilibration of the ethanol between the blood and tissue, blood samples were collected and analyzed. From the blood alcohol concentration profile that they plotted, BAC versus time, they extrapolated the best fitting line through the points on the graph back to time zero. From this they determined Widmark's C_o and $ß_{60}$. From Table 2 which shows their results, it is clear that, with the exception of dog 55, the value of $ß_{60}$ increased by about 17% every time the dose is doubled.

Figure 4 shows that while $ß_{60}$ increases with dose, the linearity of the line is not altered, and the value for $ß_{60}$ remains constant over the whole blood ethanol concentration range. In other words, it is not the concurrent blood ethanol concentration which alters the elimination rate but rather the initial dose.

Eggleton - 1940

By 1940, the consensus of scientific opinion seemed to be that ethanol is eliminated from the body at a reasonably linear rate; that this rate is independent of the concomitant blood ethanol concentration; and that while there are great interpersonal differences in elimination rates, individual elimination rates do not vary significantly from day to day.

Eggleton believed that these conclusions were not unequivocal; that

Table 2.

Effect of Dose on the Hourly Rate of Decline (mg%/hour) in the Blood Alcohol Concentration of Dogs

Dog	Dose of Alcohol (ml/kg)					
	1	2	3	4	6	1 ml after 4 or 6
51	11.4	13.8		14.4		
52	10.8	13.2		15		16.2
53	12.6	15		18		
54	11.4			18.6		
55		18		18		
57	12		13.2			
58	19.2		23.4		27	27
511				16.8	21.6	
514				20.4	29.4	
5BR	13.2			21.6		

Adapted from Newman, Lehman and Cutting (1937) page 59

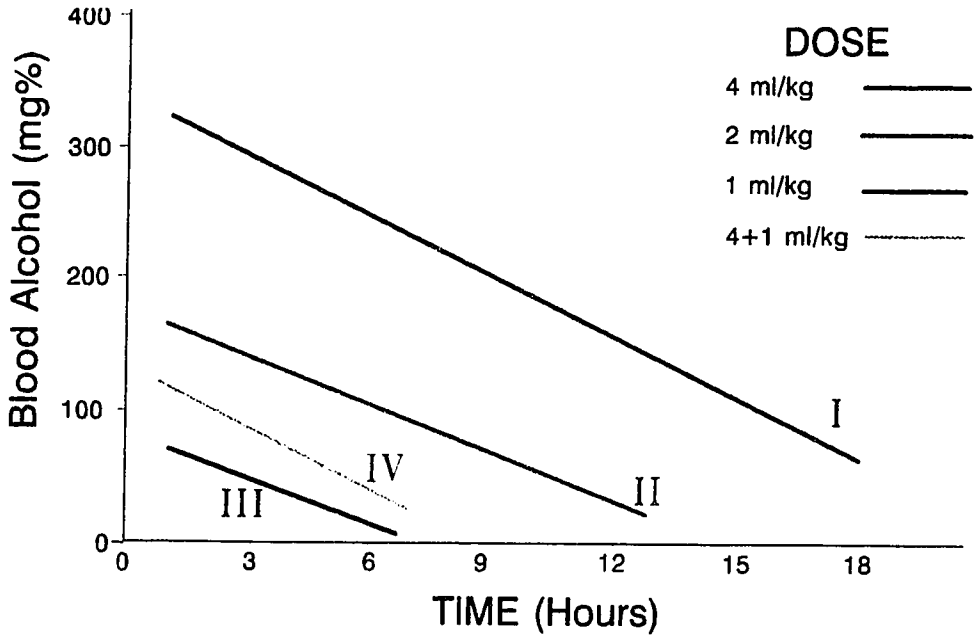


Figure 4. Lines I, II, and III are the elimination phases after doses of 4, 2 and 1 ml/kg respectively, were intravenously injected into dog 52. Once the blood ethanol concentration for line I (4 ml/kg) had declined to 60 mg%, a subsequent dose of 1 ml/kg was injected, resulting in line IV.

Adapted from Newman, Lehman and Cutting (1937) page 60

attention needed to be directed toward answering basic questions concerning four factors which may alter the metabolic rate of ethanol[24]. He identified these factors as the concentration of ethanol, the size of the liver, previous drinking, and the amino acid, alanine. In designing his experiments, he recognized that because elimination rates vary so greatly within a given population, either he must use a large number of subjects and controls and treat the results statistically, or he would have to allow each subject to act as his own control. He chose the latter approach.

The Concentration of Ethanol as a Factor

In the first of three experiments dealing with the effect of the blood ethanol concentration on the rate of metabolism, Eggleton used both dogs and cats. Each animal was injected with an initial dose of ethanol. The subsequent rate of decline was then measured for a period of time before a second, higher dose of ethanol was injected into the same animal. Again the rate of decrease in the plasma ethanol concentration was measured and compared to the first rate. In all cases, the higher dose of ethanol resulted in a faster clearance of ethanol from the plasma than the lower dose. Figure 5 shows the results for one such animal.

In the second experiment for this series, Eggleton followed the course of elimination over an extended period of time to see if a declining plasma ethanol concentration was associated with a change in the metabolic rate. Under similar conditions, Haggard and Greenberg[25] had previously observed a 17% per hour decrease in the metabolic rate. In contrast Eggleton noted only a gradual reduction in the metabolic rate with decreasing plasma ethanol concentrations. In fact the change over the course of 2 - 3 hours was so small that it appeared that the decline was linear.

The third experiment involved two stages. During the first stage, changes to the plasma ethanol concentration were observed while ethanol was being infused into the animal at a constant rate. Once a pattern of decline had been established, a single injection of ethanol was given to increase the ethanol concentration in the body. This marked the beginning of the second stage wherein the change in the plasma ethanol concentration was again monitored while ethanol continued to be infused at the same rate as in stage one. Eggleton then compared the change in the plasma ethanol concentration in stage one to that in stage two.

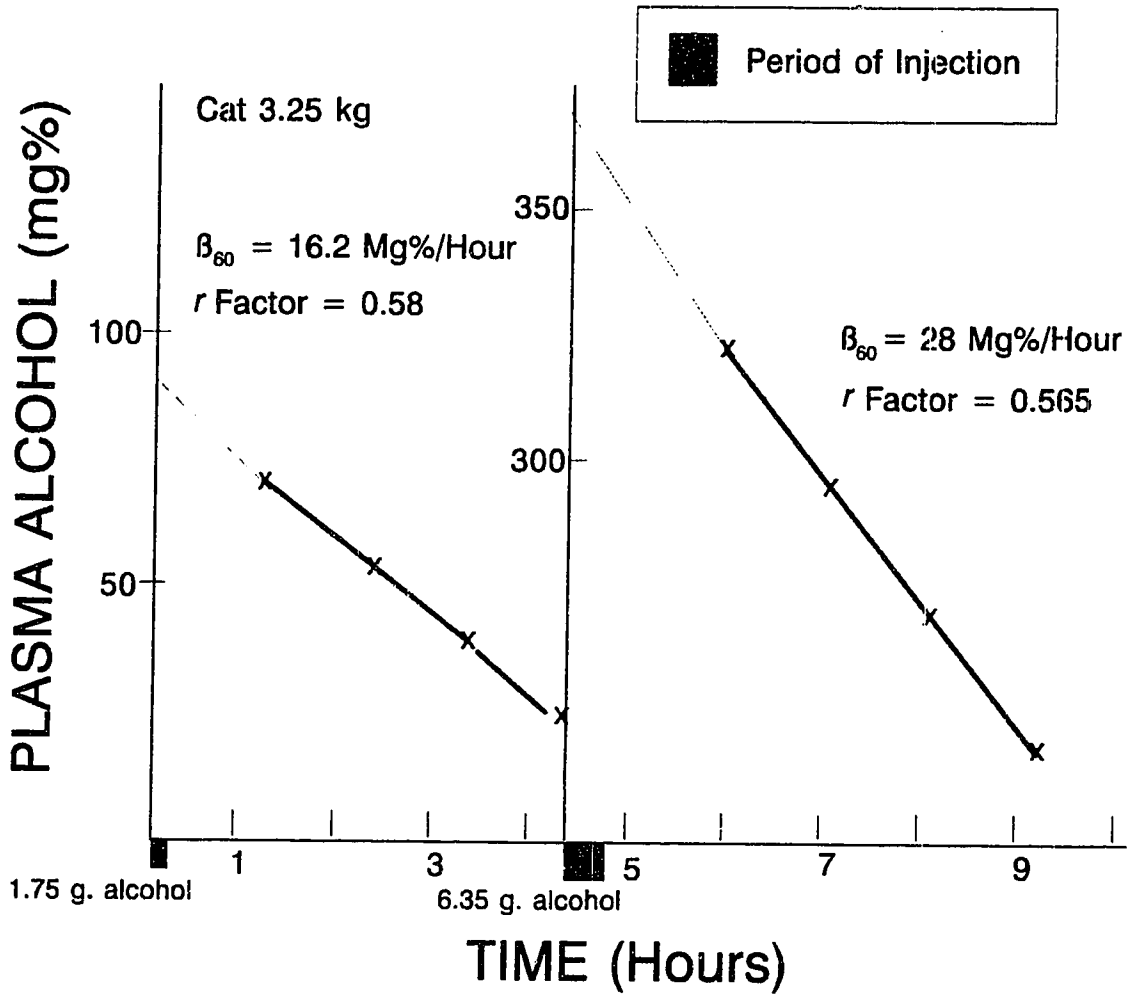


Figure 5. An initial dose of 1.75 grams of alcohol was injected into a cat. Injection periods are represented by red boxes along the abscissa. A diffusion period followed the injection. Four blood samples were collected and analyzed for their plasma alcohol concentration (mg%). The rate of decline for the plasma alcohol concentration during this first stage was -16.2 mg% per hour. Once the plasma alcohol concentration had descended to about 24 mg%, a second dose of 6.35 grams of alcohol was injected followed by a period of diffusion. During this second stage, four blood samples were collected and analyzed. The rate of decline for the plasma alcohol concentration during this second stage increased to -28 mg% per hour. The blue dashed lines represent the extrapolated plasma alcohol concentration back to the beginning of the alcohol injection period. Widmark's *r* factor is given for each of the two stages.

Taken from Eggleton (1940) page 241

Figure 6 shows that during the first stage the infusion rate exceeded the elimination rate, resulting in a net increase in the plasma ethanol concentration. However, even though the rate of ethanol infusion remained constant during the two stages, the plasma ethanol concentration was on a continuous decline during the second phase. It appears that the injection of the ethanol at the beginning of the second stage somehow triggered an increase in the elimination rate.

Eggleton showed the rate of ethanol metabolism within a single animal could be altered by a change in the concentration of ethanol in the animal's body. However, he admitted that when he attempted to compare the different elimination rates of the different animals, he was not able to provide a statistically significant relationship between ethanol concentration and the rate of elimination. This did not appear to be of much concern to him since he argued that the elimination rates varied so widely between one animal and the next in any event. He noted that "The relationship observed in any one animal was masked by some factor or factors varying in different animals, of which one was the actual body weight. The metabolic rate of ethanol tended to be lower in the larger animals."[26]

The Liver as a Factor

In two series of tests with cats and dogs, Eggleton found that there was a better correlation between elimination rates and the size of the liver than with the weight of the animal. He also found that animals suffering from jaundice eliminated ethanol at lower rates than the normal animals. However, until it is possible to establish *in vivo* the weight of a human subject's liver, Eggleton suggested that it is probably just as accurate to continue using human body weight as the criterion for measuring elimination rates, *i.e.* milligrams of ethanol per kilogram per hour (mg/kg/hour).

Tolerance as a Factor

In this series of experiments, Eggleton fed sixteen cats milk containing 5% by weight ethanol in an attempt to make these animals "ethanol tolerant". This ethanol-milk mixture was the only source of food for these cats. Nine of the sixteen cats refused to drink the milk and began to lose weight steadily for a few days before they were returned to a normal diet and excluded from the experiment. Five of the remaining 7 cats lost from 5% to 28% of their body weight while on the ethanol-milk diet. The other two maintained their body weights.

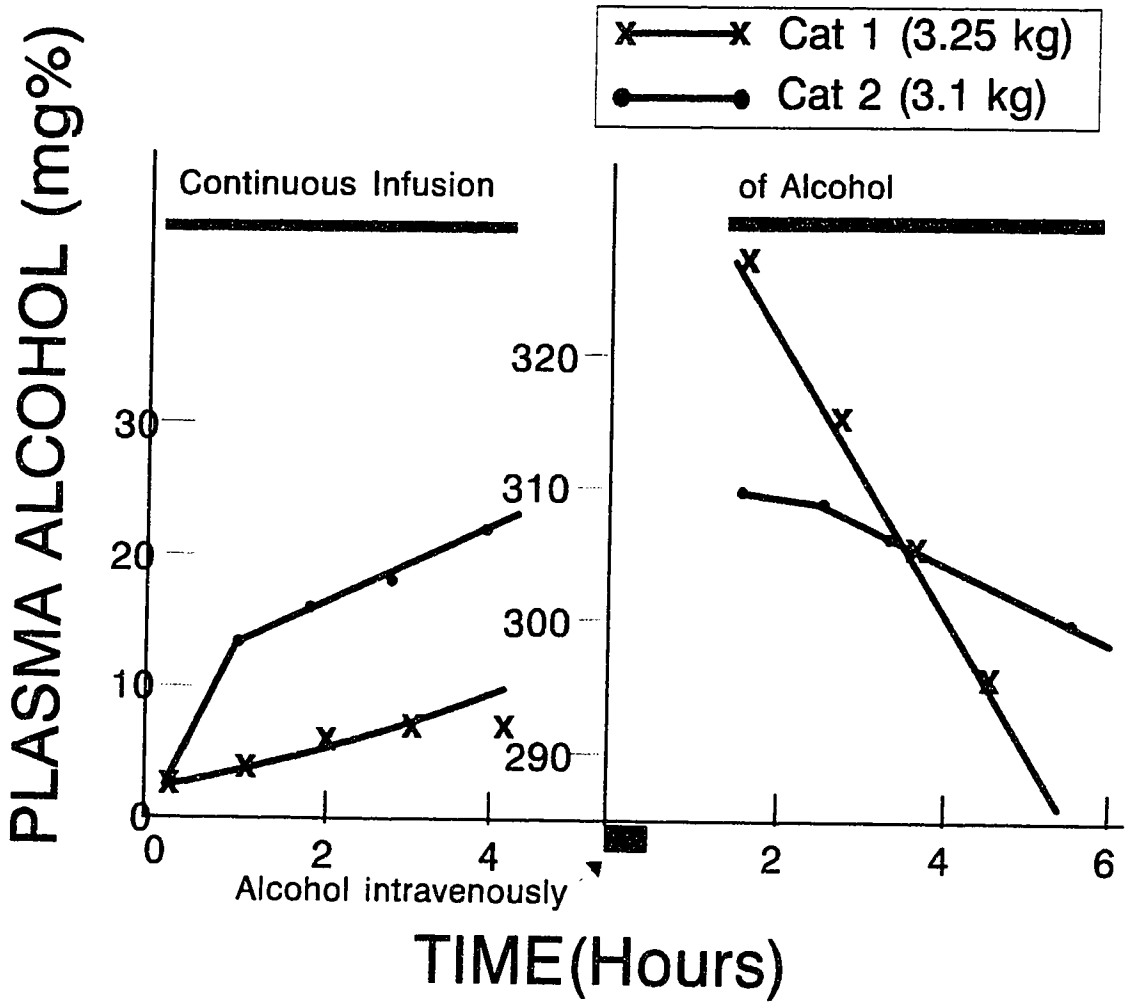


Figure 6. Ethanol was infused at a constant rate in two different cats. For cat 1 (green "X") (3.25 kg) the infusion rate was 270 mg/kg/hour; for cat 2 (blue circles), the rate was 455 mg/kg/hour. During the first stage, the plasma alcohol concentration rose in each of the two cats. However, immediately following the intravenous injection of alcohol at about the three hour time (black box), the plasma alcohol concentration fell even though the infusion rate of alcohol remained the same as for the first stage.

Taken from Eggleton (1940) page 243

The elimination rates for the "tolerant" cats were compared to the group of cats who had not been previously exposed to any ethanol and therefore acted as the control group. When the comparison was made based on body weight, there was no significant difference between the control and "tolerant" cats. However, when the comparison was based upon liver size, the "tolerant" cats eliminated the ethanol at between 50% and 80% the rate of the control cats. Eggleton reasoned that "...in the 'tolerant' animals an upper metabolic limit is reached which cannot be increased by further increase in ethanol concentration."[27].

Food in the Stomach as a Factor

Mellanby was probably the first to show that the presence of food in the stomach results in a lower blood ethanol concentration than when the same amount of ethanol is consumed on a empty stomach[28]. As well, he showed that some types of food, *e.g.* milk, had a greater effect than did cheese or meat. It appeared that these lower blood ethanol concentrations could be explained by a combination of delayed absorption of the ethanol into the blood and a possible increased metabolic rate. Leloir & Muñoz (1938) showed that when the amino acid alanine and pyruvic acid, a metabolite of carbohydrate metabolism, were added to liver slices, there was an associated increase in the metabolic rate of ethanol[29].

Eggleton, again using cats, injected ethanol into the animals and measured both Widmark's r factor and β. He found that as long as he was infusing alanine into the animals, the elimination rate was increased while maintaining a constant r factor. He also found that the elimination rate returned to control conditions once the alanine infusion was stopped. Table 3 shows his results.

Lundquist and Wolthers - 1958

Forty five years after Michaelis and Menten[30] published their paper on saturation enzyme kinetics, Lundquist and Wolthers[31] became the first to describe the elimination of ethanol from the human body as following Michaelis-Menten kinetics.

Lundquist and Wolthers begin their monograph by recognizing that the elimination of ethanol from the human body is due to the combined effects of excretion and metabolism. Removal of ethanol through the kidneys and lungs was identified as the major contributor to excretion. Since the amount of ethanol excreted via the lungs is proportional to the concentration of ethanol in the

Table 3.

Effect of Infused Alanine on β_{60} in Cats

Weight of Cat (kg)	Total Alanine Injected (g)	Widmark's *r* Factor	β_{60} (mg%/hour)	Plasma Alcohol Concentration (mg%)
2.3	0	0.52	20.7	250-300
	2	-	27.9	200-250
2.5	2	0.605	26.1	40-80
	0	0.59	22.2	110-230
3.12	2	0.65	19.2	80-130
	0	0.64	19.2	130-180
2.26	0	0.59	24.9	70-130
	5	0.59	47.4	130-170
2	0	?	28.8	150-200
	5	0.61	36	150-200
2.2	5	0.62	31.8	50-130
	0	0.62	22.5	80-120
2.8	0	0.645	21	80-130
	5	<0.68	27	110-140
2.28	0	0.64	25.5	100-150
	6	0.65	35.4	80-150

Adapted from Eggleton (1940) page 250

blood, the process must follow first order kinetics. They reasoned that at a blood ethanol concentration of 50 mg%, the concentration of ethanol in the alveolar breath will amount to about 0.25 mg/litre. With a ventilation rate of 500 litres per hour this would amount to an elimination of ethanol from the lungs at a rate of 125 mg of ethanol per hour, or about 2% of the total amount of ethanol eliminated. Similarly, with a urine flow of 2 ml per minute, about 80 mg per hour of ethanol will be excreted via the kidneys which accounts for less than 1% of the total ethanol eliminated. Therefore, at a blood alcohol concentration of 50 mg% or less, of all ethanol eliminated, less than 3% is excreted as unchanged ethanol via the lungs and kidneys. Naturally, with higher blood ethanol concentrations, the excretion rate will increase. Since Lundquist and Wolthers pointed out that the metabolic rate should remain constant, a greater proportion of ethanol will be excreted at the higher blood ethanol concentrations. In fact, for blood ethanol concentrations of about 300 mg%, as much as 15% of all ethanol eliminated will be excreted via the lungs and kidneys. This may in part explain the observations of Neuman[32], Eggleton[33], and others who observed that the apparent rate of ethanol elimination increased with increases in the initial dose given.

At blood ethanol concentrations less than 100 mg%, the amount of ethanol excreted is relatively low. Therefore, Lundquist and Wolthers concluded that elimination is essentially a function of metabolism. With the exception of Greenberg and Haggard, it was believed that the elimination of ethanol is linear. Lundquist and Wolthers argued that this could not be since at some point the blood ethanol curve must gradually approach the zero base line. Therefore, rather than the elimination being simply zero order kinetics as originally proposed by Mellanby[34], Lundquist and Wolthers argued that ethanol metabolism probably conforms to Michaelis-Menten kinetics as do most other biological processes. Liver ethanol dehydrogenase (ADH) was assumed to be the rate limiting enzyme.

Lundquist and Wolthers assigned V_{max} to designate the maximum capacity of the ADH to metabolise the ethanol. They warned that this may not be the true maximal velocity since the concentration of coenzymes in the living organism may not necessarily be optimal. If C is the serum ethanol concentration, then:

$$-\frac{dC}{dt}=\frac{V_{max}C}{C + K_M} \qquad (12)$$

This can be integrated to give the following elimination curve:

$$V_{max}t = (C_0 - C) + K_M \ln \frac{C_0}{C} \qquad (13)$$

To graphically solve for V_{max} and K_M this can be rearranged to produce:

$$\frac{(C_0 - C)}{t} = V_{max} - \frac{K_M}{t} \ln \frac{C_0}{C} \qquad (14)$$

Figure 7 shows that a plot of $(C_0 - C)/t$ as a function of $1/t \ln C_0/C$ will have a slope of $-K_M$ and an intercept of V_{max} when $1/t \ln C_0/C = 0$. This is the Eadie-Hofstee plot[35].

There are a number of advantages to this plot. The main advantage of this transformation of the Michaelis-Menten equation over the Lineweaver-Burk plot is that it provides a better distribution of data points along the length of the plot rather than a grouping of data points at either end of the plot..

A second advantage to using the Eadie-Hofstee plot is that fairly robust values for K_M and V_{max} can be achieved even when marked scattering of the data points occurs.

In testing to see if ethanol elimination adheres to Michaelis-Menten kinetics, Lundquist and Wolthers made 6 assumptions.

1. *Elimination of the ethanol takes place uniformly in a system, which is equivalent to a homogeneous phase of constant volume.* This in general is probably not true since the metabolism of the ethanol takes place mainly in the liver through which the blood from the rest of the body circulates.
2. *It is assumed that there is diffusion equilibrium between the ethanol in the blood and the ethanol in the surrounding tissues and that the cubital blood sample collected reflects this equilibrium.* Clearly, the blood upon leaving the liver will have a lower ethanol concentration than when it entered the organ. The question is, is there sufficient time for an equilibrium between the blood and the surrounding tissues to be re-established before the blood reaches the cubital vein from which they collect their blood samples.
3. *It is assumed that the ethanol consumed is completely absorbed before the testing, and that there is an equilibrium between the ethanol in the watery tissues of the intestine and the circulating blood, and that ethanol is not continually being re-introduced to the system by bacterial activity in the intestines or elsewhere.* In other words, all the ethanol that is going to be absorbed has been absorbed, and that there is not a fresh supply continually being generated by bacterial activity.

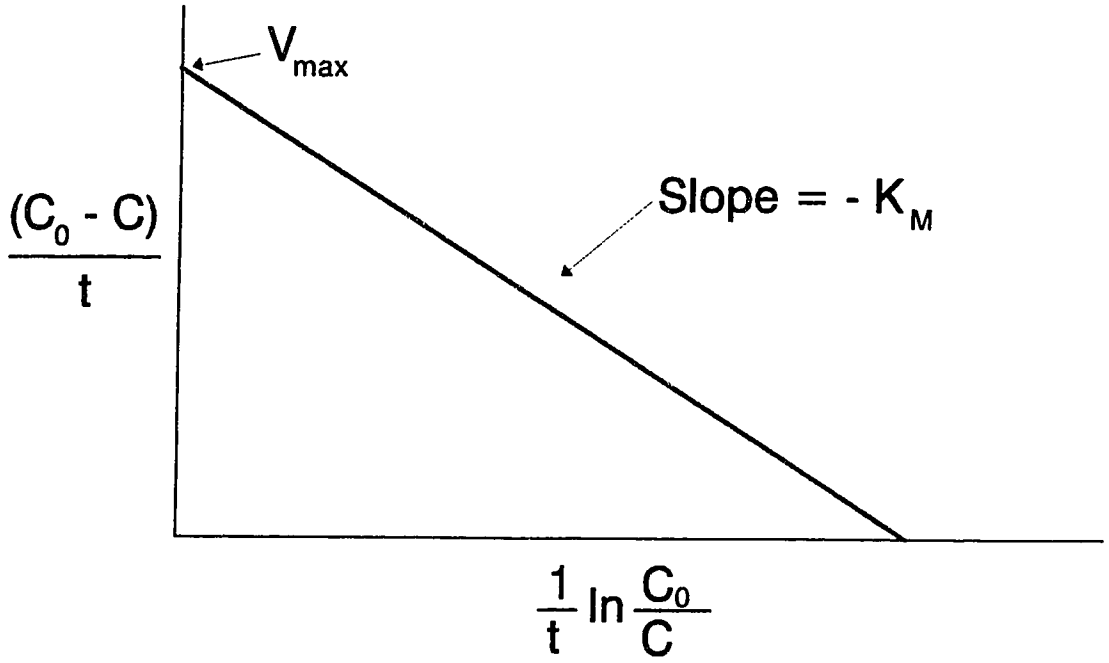


Figure 7. The Eadie Hofstee plot graphically providing values for K_M and V_{max}.

4. *Once the acetaldehyde has been formed as a result of the first step of ethanol metabolism, the acetaldehyde is so rapidly removed that it does not inhibit the ongoing reaction between the ADH and the ethanol.*
5. *Only one process is limiting the rate of disappearance of the ethanol over the whole concentration range investigated.* The possibility does exist that there may be different metabolic processes, *i.e.* the cytochrome P-450 system, or the microsomal enzyme oxidase system, which become involved at some concentrations of blood ethanol. If this combined effect does occur, then it may be very difficult to analyze the kinetics involved.
6. *There is no change in the internal factors affecting alcohol metabolism such as* pH, *ionic concentration or the concentration of coenzymes.*

Lundquist and Wolthers concluded that if all of these assumptions are true, then the data points on the Eadie-Hofstee plot should all fall along a straight line.

To test their hypothesis that ethanol elimination kinetics conform to Michaelis-Menten kinetics, Lundquist and Wolther used 10 subjects aged 20 to 40 years. Each subject fasted for at least 12 hours before drinking 0.5 grams of ethanol per kg body weight. The first blood sample was collected from the antecubital vein 2 hours after consumption ceased. Sampling continued every half hour for the next 5 hours. The blood samples collected were allowed to separate, and the plasma drawn off and analyzed using the yeast ethanol dehydrogenase assay. Control plasma ethanol concentrations collected immediately prior to the drinking and analyzed by this method revealed ethanol concentrations of 0.31 to 2.1 μg/ml with an average of 1.2 μg/ml (0.0012 mg% plasma ethanol concentration). They believed that this residual ethanol concentration is due to bacterial fermentation in the intestinal flora.

Lundquist and Wolthers then used the Eadie-Hofstee plot to get values of V_{max} and K_M for each subject. To avoid subjective influence, linear regression analysis was used to fit the straight line to the data points on the plot. The values for K_M ranged from 1.52 to 3.13 mM with a mean of 2.03 mM. This appears to be in fair agreement with the K_M for ADH of 1.75 mM which was measured *in vitro* under physiological conditions at a temperature of 33°C and a *p*H of 7.1[36]. The values of V_{max} ranged from a low of 14.9 to 25.3 mg% plasma ethanol per hour. The average value for V_{max} was 22 mg% per hour.

In addition to assaying for the ethanol concentration, Lundquist and Wolthers measured the acetaldehyde concentration in the plasma samples collected. They found that the concentration of acetaldehyde in the control samples (*i.e.* taken before drinking commenced) did not differ significantly from the samples taken subsequent to the drinking. In both sets of samples, the concentration never exceeded 0.03 mg%. Therefore, it can be reasonably assumed that, consistent with assumption 4, there was no accumulation of acetaldehyde post drinking which might have inhibited ethanol metabolism.

Using the above techniques, Lundquist and Wolthers tested glucose and fructose as possible accelerants of the rate of ethanol metabolism[37]. Using the same protocol as previously discussed, 5 human subjects drank 0.5 g of ethanol per kg body weight. Two hours later, they were fed 22 g of fructose or glucose dissolved in water over a 30 minute period. There was no significant difference between the K_M values calculated prior to the consumption of either the glucose or the fructose, and those after consumption of the sugar. This was not the case however with the V_{max} values. Fructose caused a 50% increase in V_{max} values, while glucose produced only barely perceptible increases.

It appears that the rate limiting step in the metabolism of ethanol is the dissociation between ADH and the reduced diphosphopyridine nucleotide. They offer 3 possible explanations for this fructose-induced increase in V_{max}.

1. *A change the ionic environment causing an increase in the dissociation constant of the ADH-DPNH complex.*
2. *An increase in the DPN concentration which would result in an increase in the steady state concentration of the ADH-DPNH complex.* The concentration of DPN could be increased by carbohydrates and products of carbohydrate metabolism which act as substrates for the various liver enzymes which oxidize DPNH to DPN.
3. *Oxidation of the ADH-DPNH complex by means of an aldehyde that reacts with ADH.* An intermediate metabolite of fructose metabolism is glyceraldehyde; glyceraldehyde is rapidly converted to glycerol by liver ADH at a rate proportional to the glyceraldehyde concentration.

Shumate, Crowther and Zarafshan - 1967

Shumate *et al.* were probably the first to use the Breathalyzer® to measure the variability of human elimination rates[38]. They were attempting to answer just two questions.

1. Does an individual's rate of elimination vary from test to test, and from day to day?
2. By how much does the rate vary from one individual to another?

Their sample consisted of 5 males and 5 females, ranging in age from 21 through 49 years, who had responded to an advertisement in the local newspaper. They were instructed to refrain from eating prior to arriving at the laboratory. Upon their arrival they ate a "standard" breakfast consisting of dry cereal, toast and coffee. Following the breakfast, a half hour was allowed to elapse before the subjects began to drink the ethanol which was mixed with either Coca-Cola™, ginger ale or orange juice. The choice of mix was left to each subject. Sufficient ethanol was consumed by these individuals to reach peak blood ethanol concentrations of 150 mg%.

Once consumption had ceased, a 5 minute period of time elapsed during which the subjects rinsed their mouths with tepid water (36.7 °C) before breath testing commenced. After that, Breathalyzer® tests were conducted every five minutes until the results indicated a zero blood ethanol concentration. This required from 6 to 10 hours, depending upon the initial blood ethanol concentration achieved and the individual's elimination rate. The Breathalyzer® was standardized using a standard ethanol and water solution as described in the Breathalyzer® manual[39].

Before stating their conclusions, Shumate *et al.* made this disclosure. "...work of this type requires a more sensitive measuring device than the standard model Breathalyzer. The normal rate of decline in blood ethanol is estimated to be just over .001% [1 mg%] for a 5 minute interval. This produces a situation in which the probable measurement error is larger than the expected change in the blood ethanol level"[40]. The inherent error of the Breathalyzer® is ± .01% (± 10 mg%). Despite this, even today both the Royal Canadian Mounted Police Forensic Laboratories and private laboratories continue to use the Breathalyzer® to measure elimination rates for official purposes.

Shumate and his co-workers also made the following observations.

1. Following consumption, each subject purged their mouth with tepid water. In spite of this attempt to rinse out any residual mouth ethanol which would add to the result of a Breathalyzer® test, breath samples collected and analyzed over the next 15 minutes were still affected by mouth ethanol.
2. Consistent with the conclusions of previous workers, excepting Haggard and Greenberg, the rate of ethanol elimination was independent of the amount of ethanol in the blood.
3. A period of instability in the blood ethanol concentration was observed for the first hours following the end of drinking. They noted that this "phenomenon" was independent of the amount consumed and the blood ethanol concentration, and further speculated that this it was due to absorption of ethanol into the blood stream. As well, they were intrigued by the rather constant rate of decline in the blood ethanol concentration during this period of time beyond which they assumed that absorption would be complete.
4. The average rate of ethanol elimination for their subjects was 17 mg% per hour, with a range of 10 mg% to 22 mg%.

The third observation ignored the comments of Widmark made at length 35 years earlier[41] in his book about this period leading up to diffusion equilibrium, and this book was referenced in Shumate's paper.

The results of Shumate and his co-workers testing with the Breathalyzer® revealed that while elimination rates do vary within a population of individuals, individual elimination rates remain constant over time and, do not change from day to day.

Vesell, Page and Passananti - 1971

A novel protocol for testing the day to day variability of ethanol elimination rates was introduced by Vessel *et. al.*[42]. Their sample consisted of 7 pairs of monozygotic (identical) twins, and 7 pairs of dizygotic (fraternal) twins. All twenty eight of these twins were in good physical health and had not received medication during the 1 month interval preceding the testing. Each twin drank ethanol (0.75 g/kg) diluted with ice water over a ten minute period. Ninety minutes later, the first blood sample was taken. Sampling continued about every half hour for the next 2½ to 3 hours. Plasma was drawn off of the whole blood and analyzed by gas liquid chromatography. Their results are shown in Table 4.

From this data it can be seen that the mean elimination rate for both identical and fraternal twins was 16 mg%/hour with a range of 11

Table 4.

Comparison of Plasma Ethanol Elimination Rates

For Paired Identical and Fraternal Twins

Gender, Age	Alcohol Elimination Rate (mg%/hour)	Intrapair Difference
Identical Twins		
F, 23	16	0
F, 23	16	
F, 61	20	0
F, 61	20	
M, 22	15	0
M, 22	15	
M, 22	11	0
M, 22	11	
M, 47	13	2
M, 47	15	
F, 35	18	1
F, 35	19	
F, 56	18	0
F, 56	18	
Fraternal Twins		
F, 57	17	0
M, 57	17	
F, 47	21	7
F, 47	14	
M, 49	24	13
M, 49	11	
F, 36	20	3
F, 36	17	
F, 54	16	4
F, 54	12	
F, 32	13	2
M, 32	15	
F, 36	11	5
F, 36	16	

Adapted from Vesell *et. al. (1971)* Page 195

to 24 mg%/hour. It should be emphasized that these elimination rates are for the loss of ethanol in plasma and not whole blood. Plasma will have an ethanol concentration about 1.14 (range is 1.09 to 1.17) times as great as the whole blood ethanol concentration[43]. Of the 7 pairs of identical twins, 5 pairs had identical elimination rates, the other two had rates that differed by less than 2 mg%/hour. In comparison, only 1 pair of the fraternal twins shared identical elimination rates. For the other 6 pairs the differences ranged from 2 to 13 mg%/hour.

Vesell and his co-workers concluded that "...the individual variation in ethanol metabolism among the 28 twins was maintained almost exclusively by genetic rather than environmental control."[44]. The question that this raises is, if the elimination of ethanol is a function of genetics and there tends not to be a difference between the rates of identical twins, does this indicate that the genetically set rate remains constant, even for the same person from one day to the next?

Vessel and his co-workers also tested the effects of chronic drinking on individual elimination rates. They chose 6 Caucasian prisoners who were in solitary confinement and had been thus confined for at least 3 months preceeding the experiment. They were fed a dose of 1 ml 95% ethanol per kg each day at 9:00 a.m. for 21 successive days. As can be seen from Table 5, no consistent change was observed in the hourly elimination rate after the 21 days: three reduced their rates and three increased their rates.

Lieber and DeCarli - 1972

Prior research by Lieber and DeCarli[45,46] had demonstrated that there existed, in addition to the liver alcohol dehydrogenase system (ADH), an hepatic microsomal system also capable of oxidizing ethanol. Lieber and DeCarli called such a system the microsomal ethanol oxidizing system (MEOS). However, one of the major problems encountered in these *in vitro* animal studies was the loss of microsomes during tissue preparation. Consequently, when the rate at which ethanol was eliminated *in vitro* by MEOS was compared to the total *in vivo* rate of alcohol oxidation, the contribution of MEOS was considered inconsequential[47]. In the present monograph, Lieber and DeCarli[48] improved their separation of the liver microsomes by decreasing the time the microsomes

Table 5.

Elimination Rates for Six Male Prisoners

Before and After Chronic Alcohol Administration

Subject	Before (mg%/hour)	After (mg%/hour)
1	19	20
2	22	26
3	20	20
4	22	18
5	12	24
6	19	18
Mean ± SD	19 ± 4	21 ± 3
Coefficient of Variance	0.184	0.148

Note: The average rate of ethanol elimination for the six prisoners did not differ significantly from before the testing to after the chronic dosing (paired *t*-test which gave $t = 0.88$ for $P > .25$).

were centrifuged. They also capitalized on the fact cytochrome P-450 is found almost exclusively in the microsomes. Therefore by measuring the cytochrome P-450 content of the hepatic homogenate and the washed microsomes they were able to correct for any loss of microsomes during the isolation procedure. In fact, when they used P-450 as the marker, they found that they were only getting about 48% recovery of the microsomes.

Chronic alcohol feeding to female Dawley rats was achieved by incorporating ethanol in a nutritionally balanced liquid diet. Control animals were fed the same liquid diet except that the ethanol was replaced isocalorically by carbohydrates. Ethanol elimination rates were determined after an overnight fast followed by the administration of the alcohol liquid diet intragastrically at a dose of 3 g/kg ethanol. Pretreatment with pyrazole inhibited ADH activity, thus revealing oxidation of ethanol by other than the ADH system.

Using their new assay procedure, Lieber and DeCarli claimed that the *in vitro* MEOS activity they measured corresponded to 20 to 25% of *in vivo* ethanol metabolism. This coupled with their finding that, even after pretreatment with the ADH inhibitor pyrazole, there was still significant ethanol oxidation occurring. They reasoned that MEOS could be involved with a significant amount of ethanol oxidation *in vivo*.

There is, of course, the concern that the pyrazole did not fully inhibit the ADH, and therefore residual ADH activity might be responsible for the persistent, albeit greatly diminished, oxidation of the ethanol. Lieber and DeCarli provide three reasons to discount such a possibility.

1. Since pyrazole is a competitive inhibitor of ADH[49] with a half life of about 14 hours, any inhibition attributed to pyrazole would be diminished with time associated with a concomitant increase in ADH activity resulting in an increased rate of ethanol elimination. From their results this clearly did not happen.
2. In the time period from 27 to 49 hours after the pyrazole pretreatment, there was a marked deceleration in the rate of ethanol elimination, and not an acceleration.
3. When the rates of elimination were plotted on a Lineweaver-Burk graph, the resulting graph was linear, which is inconsistent with incomplete inhibition of the ADH. In other words, the K_M value should have decreased to approach the K_M value of non-inhibited ADH.

The second part of their argument provided five reasons why they believed the ADH was completely and competitively inhibited by

the pyrazole, and therefore could not possibly be responsible for the residual oxidation of the ethanol following pyrazole pretreatment.

1. Under controlled conditions, the blood ethanol clearance rate was 104 µmol/min/kg. However the maximum clearance rate was reduced following pyrzole pretreatment to 31 µmol/min/kg, similar to the *in vitro* V_{max} for MEOS of 29 µmol/min/kg.
2. Not only was the elimination rate markedly reduced, but the duration of the elimination phase of the alcohol concentration profile was also shortened. Normally under controlled conditions, the pseudolinear phase extends down to about 4 mM with an apparent *in vivo* K_M of 2.7 mM. Following pyrazole treatment, the pseudolinear phase was shortened with an apparent K_M of 8.8 mM which is consistent with the K_M for MEOS *in vitro* of 8.8 mM; significantly higher than that for ADH.
3. Chronic ethanol feeding caused the rate of the blood ethanol elimination to increase from the control value of 119 µmol/l/min (32.8 mg%/hour) to 174 µmol/l/min (48.0 mg%/hour). This increase in rate is probably due to two factors. The first is increased MEOS activity. The second is a possible increased ADH activity resulting from increased MEOS activity which in turn increases the turn over of the NADPH-NADP (oxidation) and the corresponding reduction couple NADH-NAD which inturn favours the reoxidation of the ADH-NADH complex.
4. MEOS activity measured *in vitro* was shown to account for almost two thirds of the increase in the ethanol elimination rate after chronic ethanol feeding.
5. A pretreatment with phenobarbital increased the *in vitro* activity of MEOS but not of ADH. In fact, the *in vivo* coexistence of phenobarbital and ethanol in the blood resulted in a decreased rate of clearance of ethanol from the blood. However, under *in vivo* conditions if following the phenobarbital pretreatment sufficient time is allotted to allow for all of the barbiturate to leave the animal and then ethanol is administered, the rate of ethanol elimination was shown to increase from 122 µmol/l/min (34 mg%/hour) to 154 µmol/l/min (42 mg%/hour).

Wagner and Patel - 1972

To test for individual day to day variability of ethanol kinetics, Wagner and Patel[50] tested a 50 year old, 63.5 kg male subject on 5 different occasions. Studies on variability from day to day conducted to this point in time dealt mainly with ß and C_0. Wagner and Patel claim to be the first to include a number of other parameters including the Michaelis-Menten parameters of V_{max} and K_M.

During the 120 days that this study was done, the subject drank ethanol socially between the five testing days, but not to more than a maximum of 1 to 2 fluid ounces per day. The subject fasted overnight prior to each testing session, and upon arrival for the testing, he was given and consumed a standardized breakfast of orange juice, toast, coffee, bacon and eggs. This was followed by an interval of 2 hours and 45 minutes (except for study number 1, for which the interval was only 45 minutes) before he was given the dose of ethanol mixed with orange juice to drink. The midpoint of consumption rather than its commencement was designated as t_0. No explanation was given for this unorthodox choice.

Following consumption, an interval of time elapsed before collection of fingertip capillary blood samples. For study number 1, this interval was 120 minutes; for studies numbered 2 to 5, this interval was 45 minutes. Once collected each blood sample was refrigerated to 4°C. Gas chromatographic analysis of these samples was completed within 24 hours of collection. The results are shown in Figure 8.

For each of the five sets of blood alcohol concentrations, non-linear regression fitting was done by numerical integration of the following Michaelis-Menten equation.

$$C_0 - C + K_M \ln(C_0/C) = V_{max}(t - t_0) \qquad (15)$$

Where C is the blood alcohol concentration in mg%, t is the time in minutes of the sampling, C_0 is the blood alcohol concentration at time t_0 and K_M and V_{max} are Michaelis-Menten parameters. Table 6 shows some of the parameters.

What is interesting to note is that there does not seem to be any correlation between dose and the C_0 values. It would seem reasonable to assume that as dose decreased, there would be a corresponding decrease in C_0. Between studies 1 and 2, even though the same dose was administered, the C_0 value for study 1 is less than one half the value when the same dose was administered for study 2. The converse is true for studies 2 and 3. It will also be noted that the K_m value for this subject was anything but constant, and fluctuated indiscrimately between 4 and 36 mg%. The values for V_{max} range between 27 and 66, all exceeding Lundquist and Wolther's range of 14.9 to 25.3 mg% per hour[51]. Even though the two blood alcohol concentration curves for studies 3 and 4 appear nearly superimposable, the respective values of K_M and V_{max} as

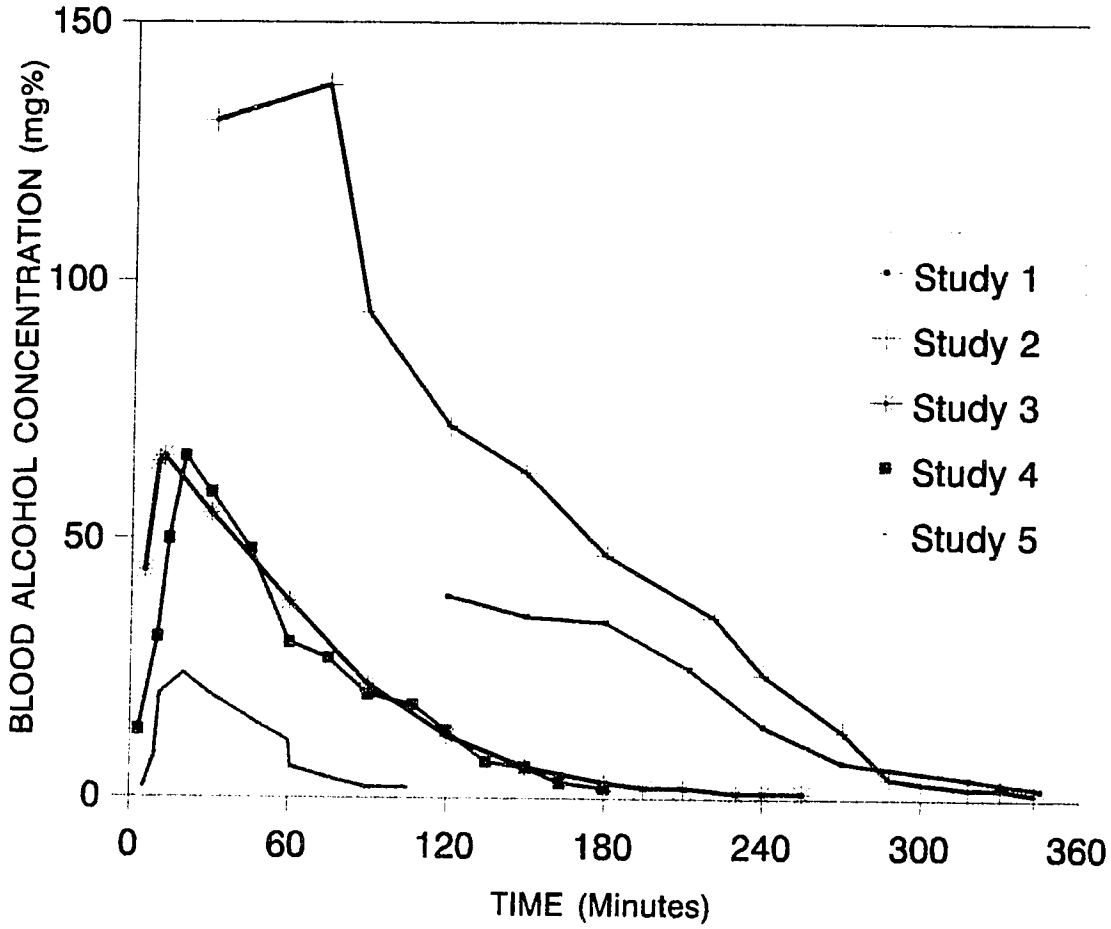


Figure 8. Capillary blood alcohol concentration curves of one subject for each of Wagner and Patel's five studies. In study one, the ethanol was consumed 45 minutes after a standardized breakfast. For each of the remaining four studies, the subject began consumption of the ethanol 2 hours and 45 minutes after eating the breakfast. The dose of ethanol consumed was 0.709 gm/kg for study one and two, 0.354 gm/km for studies three and four, and 0.177 gm/kg for study five.

Table 6.

Michaelis-Menten Parameters For Male Subject

Administered Alcohol on Five Different Days

Study	Interval (days)	Dose (g/kg)	Time Values Used (min)	C_0 (mg%)	K_M (mg%)	V_{max} (mg% /hr)	k_0 (mg% /hr.)	Coefficient of Determination	
								r^2	Corr.
1	0	0.709	180 - 345	34	14	-30	-17	-0.997	-0.997
2	19	0.709	120 - 315	73	4	-27	-24	-0.999	-1
3	28	0.354	30 - 255	55	36	-66	-32	-0.999	-1
4	40	0.354	75 - 180	27	9	-28	-17	-0.996	-0.994
5	120	0.177	17 - 105	25	5	-31	-22	-0.992	-0.992

Note: Under the heading Coefficient of Determination, the r^2 values refer to the goodness of fit for the calculated blood alcohol concentration value and the observed blood alcohol concentration values when non-linear regression analysis is used. The *Corr.* values relate to the goodness of fit between observed and calculated blood alcohol concentrations when linear regression analysis is used.

Table 7.

The Mean, Standard Deviation and Coefficient of Variance for Four Kinetic Parameters

Parameter	Mean	± SD	Coefficient of Variance (%)
C_0 (mg%)	42.5	20.8	48.9
k_0 (mg%/hour)	22.6	6	26.7
V_{max} (mg%/hour)	36.4	13.3	97.3
K_M (mg%)	13.6	16.5	45.3

Note: Wagner and Patel's subject was tested on five different days (Table 6) under dissimilar conditions. For study one, 45 minutes elapsed between the consumption of food and the drinking of the alcoholic beverage. For the remaining four studies, this interval was increased to 2 ¾ hours. The dose of ethanol given ranged from 0.177 g/kg to 0.709 g/kg depending upon the day. Therefore, the variability of the kinetic parameters shown must be viewed in this context of non-standardized testing conditions.

shown in Table 7 are anything but similar. There is considerable variance in the kinetic parameters of C_0, k_0, K_M and V_{max}.

On page 74 of their monograph, Wagner and Patel explained that,

> "The wide variation in the estimated parameters K_m and V_{max} in the same subject from one time of administration to the next surprised us, and we deemed the data worthy of reporting. Despite apparent similarity of the time courses of blood alcohol concentrations, such as following the two 30 ml. doses of 95% alcohol, quite different K_M and V_{max} values were estimated by the computer fitting. This is not unusual in nonlinear pharmocokinetics since with three parameters, C_0, K_M and V_{max}, ... there are a large number of combinations which can provide a C,t curve having a very similar time course."

In addition to the variability introduced by the computerized non-linear regression analyses, Wagner and Patel introduced other variables such as dose, and the interval of time following the ingestion of food and the commencement of drinking.

It is also interesting to note that in terms of k_0, the intersubject coefficient of variation that Vesell *et al* derived for their six prisoners (see Table 5.) was 0.184 before chronic dosing, and 0.148 after chronic dosing, compared to Wagner and Patel's intrasubject coefficient of variation of 0.267. As Wagner[52] acknowledged in a subsequent paper,

> "Thus these data tend to indicate that *intra*subject variation of the apparent zero-order rate of ethanol metabolism is greater than the *inter*subject variation. This does not appear to be theoretically sound for a large population, but for the small amount of data available one obtains that answer."

In light of this, it is very difficult to determine whether the day to day variability in absorption and elimination rates of ethanol shown by Wagner and Patel is due to the protocol used, the methods used to analyze their data, and/or the individual subject.

Wagner - 1973

The major difficulty with Michaelis-Menten kinetics is fitting a non-linear regression line to the data of an ethanol concentration versus time curve. Probably one of the first to employ computers to the task was Wagner in 1973[53]. He integrated the Michaelis-Menten equation between the limits of C=0 and C=C_0 (C_0 is the concentration at the beginning of the elimination phase of the concentration versus time curve) and obtained the following equation (equation 16):

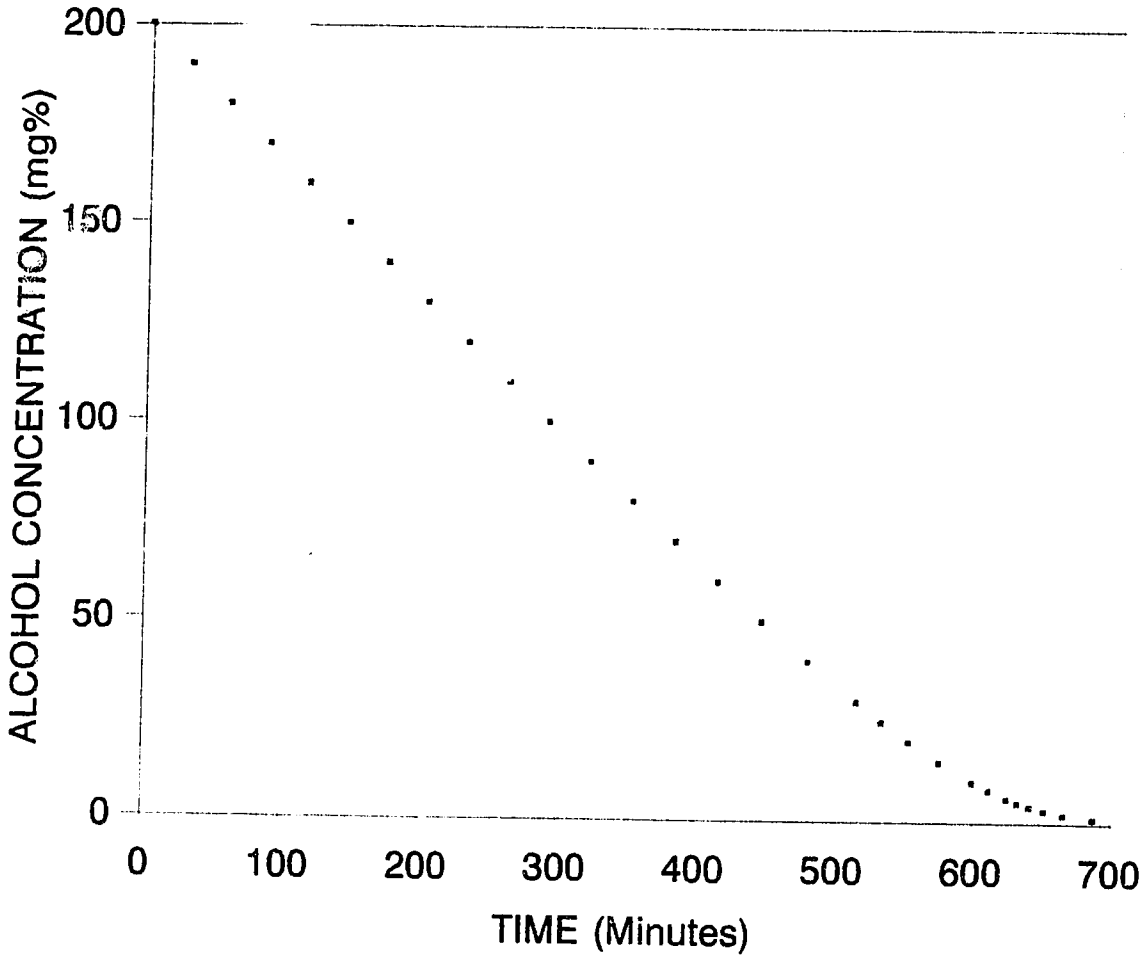


Figure 9. Ethanol concentration versus time curve employing computer generated data. Values of the parameters used in equation (16) were $K_M = 10$ mg%, $V_{max} = 0.367$ mg% per minute.

$$C_0 - C + K_M \ln(C_0/C) = V_{max} t \qquad (16)$$

where $t = 0$ when $C = C_0$. He then generated data using this equation to produce a chart similar to Figure 9. This figure shows the typical "hockey stick" shape with the upper segment of the curve appearing linear.

Preliminary Estimates of V_{max} and K_M

Fortunately there are a number of computer software programs available which will assist in fitting the best fitting trend line to these data points by non-linear regression. However, initial estimates of the two parameters V_{max} and K_M are required by these programs before they can begin to fit the line to the data points.

Method 1

Lundquist and Wolthers[54] suggested one graphical method of making this first approximation. Figure 10 uses the computer generated data from Figure 9, and formula (14) to provide the initial estimates for V_{max} which is the y intercept and K_M equal to the slope. The estimated values for V_{max} and K_M using this method are 0.368 mg% per minute and 9.53 mg%. The data points in Figure 9 were rounded off to the nearest whole number, leading to a small deviation from the theoretical values. Consistent with the warning of Lindquist and Wolther, it can be seen from Figure 10 that these small deviations in rounding off from the theoretical values result in marked scattering of the points in Figure 10.

Wagner introduced two further methods for providing initial estimates for V_{max} and K_M.

Method 2

The first of these methods makes uses of two equations with two unknowns. Three sets of data points are chosen from the ethanol concentration versus time curve. The first point should be close to the beginning of the pseudolinear elimination phase. The second point should be at or near the position where the pseudolinear decline breaks into the "foot" of the curve, with the final point near the end of the "foot" section. The respective concentrations and

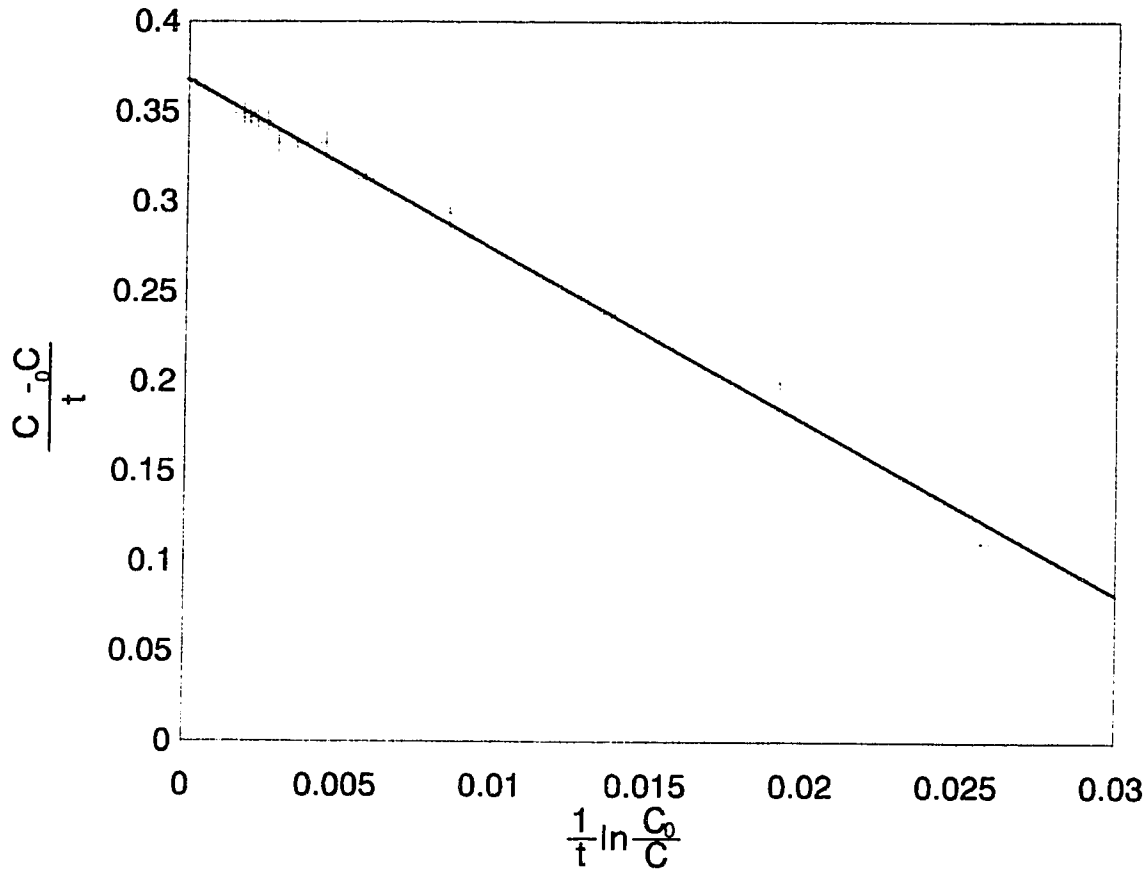


Figure 10. Using Wagner's Method 1 to provide an initial estimate of V_{max} and K_M. The plot of $(C_0 - C)/t$ versus $t^{-1} \ln (C_0 - C)$ for 12 pairs of data points shown in Figure 9 has a slope equal to $-K_M$ while the y intercept equals V_{max}.

times are then entered into the following two equations:

$$C_1 - C_2 + K_M \ln(C_1 - C_2) = V_{max}(t_2 - t_1) \quad (17)$$

$$C_1 - C_3 + K_M \ln(C_1 - C_3) = V_{max}(t_3 - t_1) \quad (18)$$

Using the data from Figure 8. $C_1 = 200$ mg%, $t_1 = 0$ minutes, $C_2 = 15$, $t_2 = 575$ minutes, $C_3 = 3$ and $t_3 = 651$ minutes

$$(200 - 15) + K_M \ln(200/15) = V_{max}(575-0)$$

$$(200 - 3) + K_M \ln(200/3) = V_{max}(651-0)$$

If one solves for V_{max} in the first equation and substitutes the value into the second equation, $K_M = 9.94$ mg% and therefore $V_{max} = 0.367$ mg% per minute.

Method 3

This graphical method is based upon the following equation which is an integrated form of the Michaelis-Menten equation.

$$\frac{C}{\Delta C/dt} = \frac{K_M}{V_{max}} + \left(\frac{1}{V_{max}}\right)C \quad (19)$$

For this equation of a straight line, C is the midpoint ethanol concentration for ΔC. Figure 11 is a plot of $C/(-\Delta C/dt)$ versus C. From Formula (19) it can be seen that the slope of the graph is equal to $1/V_{max}$ and the y interecpt equal to K_M/V_{max}. Once the data has been plotted, linear regression is used to fit the best line to the data. This method provides a preliminary value for $V_{max} = 0.367$ mg% per minute, and $K_M = 10.1$ mg%.

Clearly all three methods (Table 8.) provide good preliminary estimates for the two Michaelis-Menten parameters of V_{max} and K_M. However, what happens to these preliminary estimates if only those data points above the break in the elimination curve are used, *i.e.* 40 mg% to 200 mg%? The estimates for V_{max} and K_M are 0.369 mg% per minute and 10.6 mg% respectively when method 2 is used. When Wagner's graphical method is used, the preliminary estimate for V_{max} is 0.367 mg% per hour, and for K_M the value is 10.0 mg%.

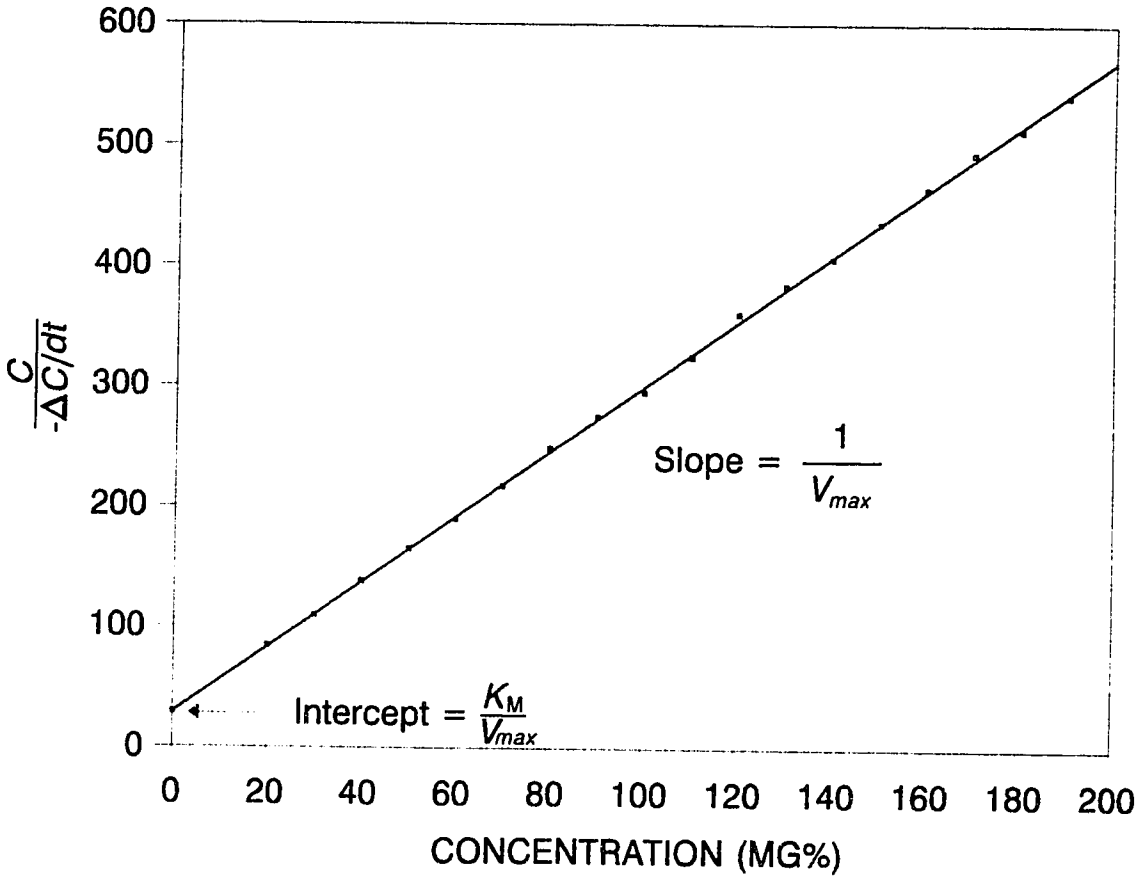


Figure 11. Using Wagner's third method to estimate K_M and V_{max}, a plot of $C/(-\Delta C/dt)$ versus C using the data from Figure 9 and Formula (19) provides a slope equal to the inverse of V_{max}, and an intercept equal to the ratio of K_M and V_{max}.

Table 8.

Comparison of the Preliminary Estimates for

Michaelis-Menten Parameters Using Three Different Methods

Method	V_{max} (mg%/minute)	K_M (mg%)
Actual Values Used to Generate the Data	0.367	10
Wagner's Method 1		
Full Set of Data	0.367	9.94
40 - 200 mg%	0.369	10.6
Wagner's Method 2		
Full Set of Data	0.367	10.1
40 - 200 mg%	0.367	10
Lundquist & Wolthers		
Full Set of Data	0.368	9.53
40 - 200 mg%	0.369	10.3

Note: The actual values for V_{max} and K_M (second row in this table) used to produce the data points for Figure 9 were 0.367 and 10 respectively. Based upon these computer generated data points, all three methods used to provide preliminary estimates of V_{max} and K_M, provide good estimates of these two Michaelis-Menten parameters.

The Pseudolinear Nature of the Elimination Phase

A plot of concentration versus time using the integrated form of the Michaelis-Menten equation (16) in a sense is bi-phasic. The initial decline appears to be linear as the concentration declines and time increases. This pseudolinear phase continues until some concentration at which the shape of the graph becomes quite curved. This raises two major questions. Is the initial decline actually non-linear, and at what ethanol concentration does the shape of the curve change?

To answer the first question, Wagner plotted the change in concentration with time ($-dc/dt$) versus time (t). From Figure 12 it can be seen that there is a continuous decrease in $-dc/dt$ as time increases. In fact, at some point the decrease becomes quite dramatic. Wagner therefore concluded that the elimination of ethanol is non-linear, and that this holds true even during the initial decline which appears to be quite linear.

Answering the second question requires a bit more reasoning. Clearly, in order for there to be a near linear decline in the changing ethanol concentration (referring to equation 16) the magnitude of $K_M ln(C_0/C)$ must be minimal compared to $(C_0 - C)$. This is achieved when $ln(C_0/C)$ is equal to less than 1. Similarly, the influence of $K_M ln(C_0/C)$ in increasing the curve of the graph is increased when $ln(C_0/C)$ has a value greater than 1. In other words, as $K_M ln(C_0/C)$ approaches 0 the linearity of the graph increases, and as $K_M ln(C_0/C)$ becomes > than 0 the linearity of the graph decreases. The breaking point between the near linear phase and the non-linear phase is when $ln(C_0/C) = 1$. This is equivalent to $C_0/e = C$ where e is the base of natural logrithms (Figure 13).

When $C > C_0/e$ then $ln(C_0/C) < 1$ and the effect of K_M is lessened as C increases; resulting in a more linear graph. Conversely, when $C < C_0/e$ the value of $ln(C_0/C) > 1$ and the effect of K_M on the magnitude of the curve of the graph increases as the ethanol concentration decreases.

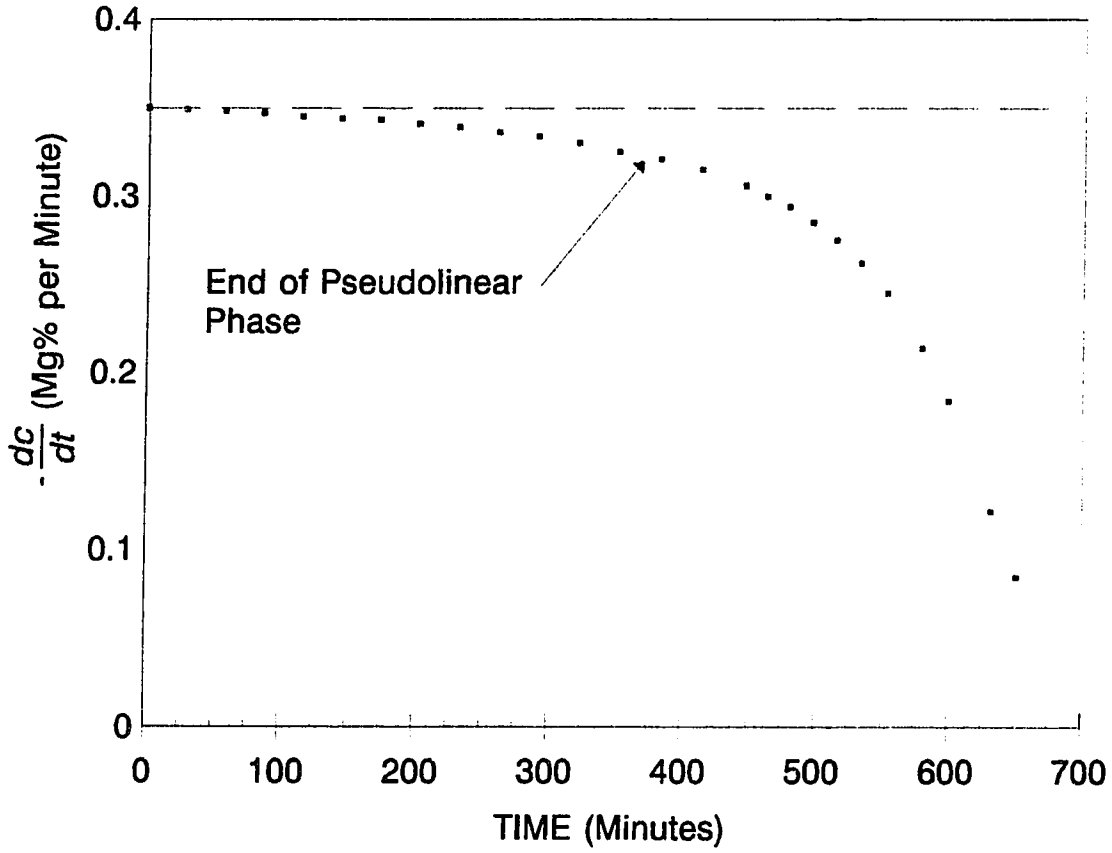


Figure 12. A comparison of the change in velocity with time. The data is taken from the computer simulated concentration versus time graph shown in Figure 9 and the Michaelis-Menten equation $-dc/dt = V_{max}\, C/(K_M + C)$. This shows a persistent decrease in the overall velocity of the elimination of ethanol, even in the apparently linear segment of the concentration versus time curve of Figure 9.

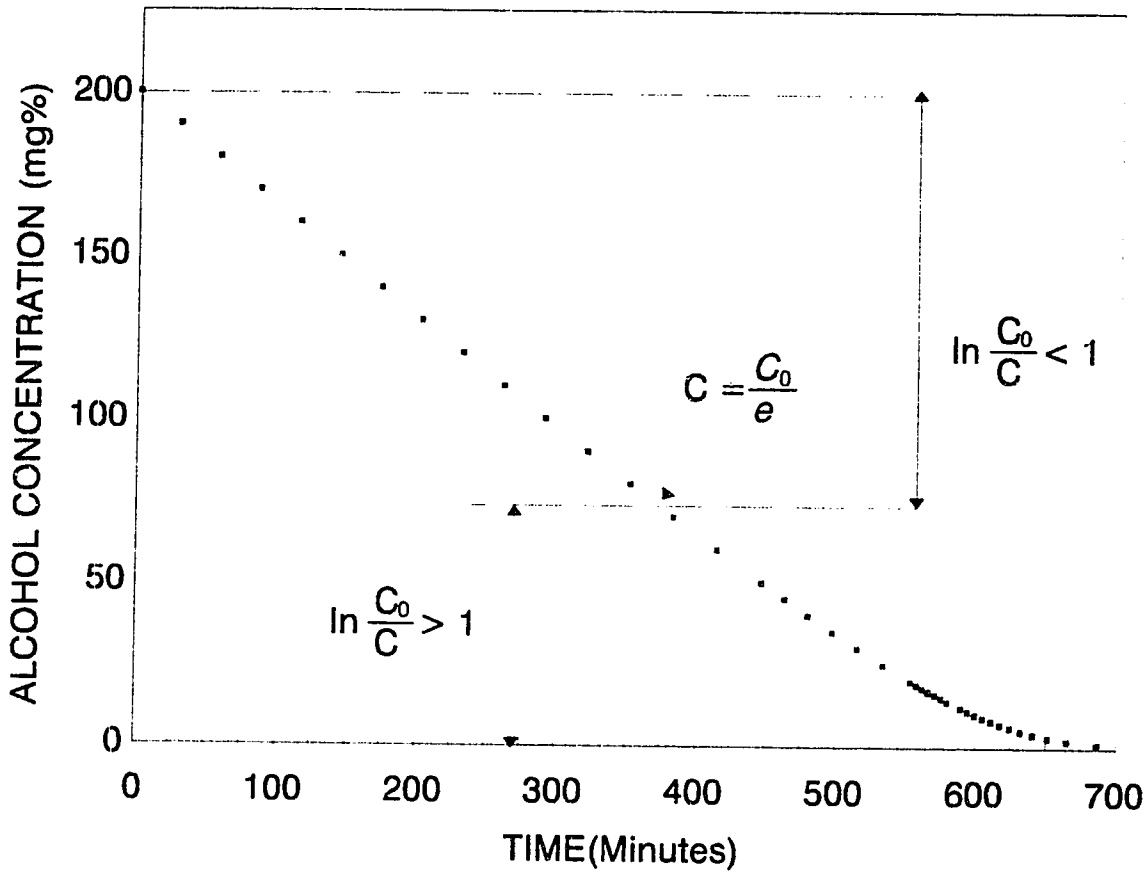


Figure 13. The effect of C on the shape of the ethanol concentration versus time graph. As $ln(C_0/C)$ decrease from 1 to 0, the graph becomes more linear. Conversely as $ln(C_0/C)$ increases above 1, the graph becomes less linear.

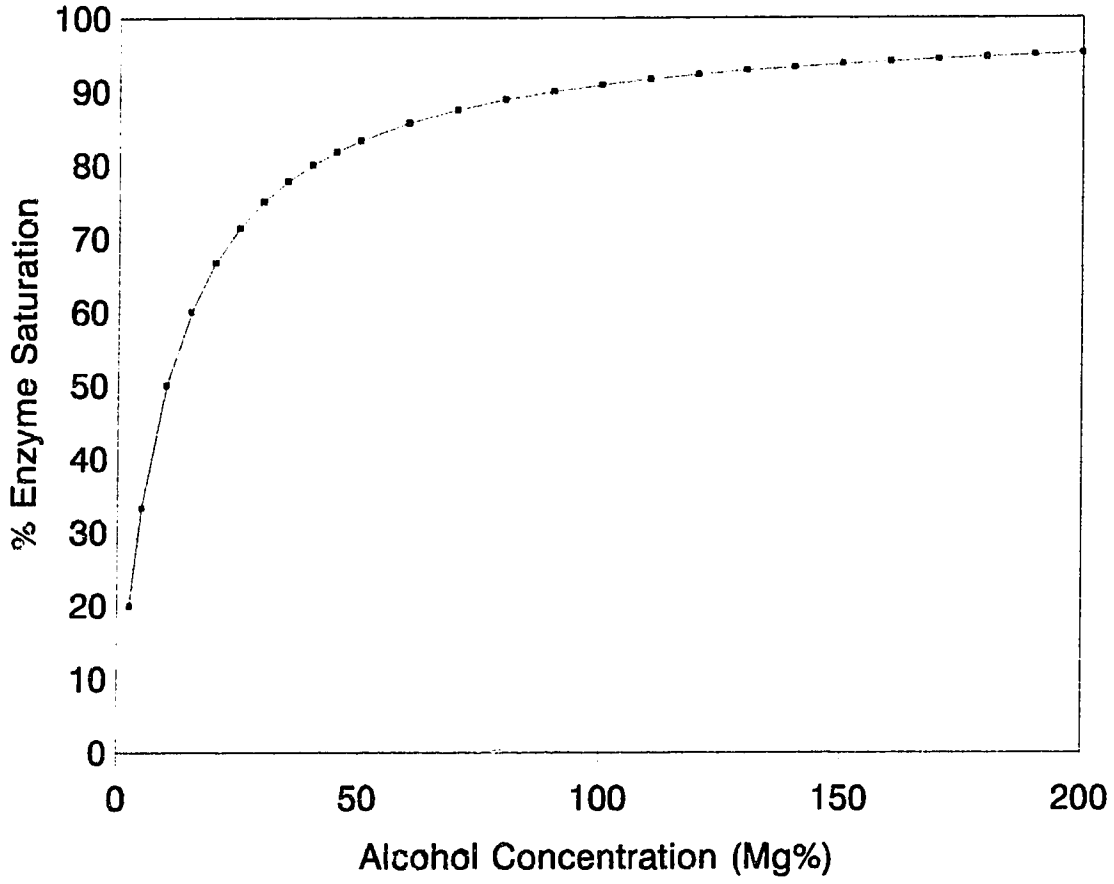


Figure 14. The percent of enzyme saturation versus the ethanol concentration. Data from Figure 9 and equation (22) were used (K_M= 10 mg%) to calculate the percent saturation of the metabolizing enzyme. Complete saturation of the enzyme only occurs as the ethanol concentration approaches infinity.

If one assigns t_{BRK} to equal the time when $C = C_0/e$, then by substitution into equation (16) one gets:

$$t_{BRK} = \frac{1}{V_{max}}\left(C_0 - \frac{C_0}{e}\right) + \frac{1}{V_{max}}\left(K_M \ln\left(\frac{C_0}{\frac{C_0}{e}}\right)\right) \quad (20)$$

BUT $$\ln\left(\frac{C_0}{\frac{C_0}{e}}\right) = 1$$

$$t_{BRK} = \frac{C_0}{V_{max}}\left(1 - \frac{1}{e}\right) + \frac{K_M}{V_{max}} \quad (21)$$

Using equation 21, the value of t_{BRK} for the data used in Figures 12 and 13 is 372 minutes.

As can be seen from Figure 14, the enzyme in question does not readily become saturated with the circulating blood ethanol. The percent saturation of the enzyme can be calculated from equation (22)

$$\% \text{ Saturation} = \frac{-dC/dt}{V_{max}} \times 100 = \frac{100\,C}{K_M + C} \quad (22)$$

Applying equation (22) to the data from Figure 9 it is clear that the enzyme never fully becomes saturated as is commonly believed. It is only when the ethanol concentration approaches infinity that the enzyme approaches complete saturation (Figure 14).

This therefore begs the question. What is the change in the saturation of the enzyme between an ethanol concentration of, say, 40 mg%, and one that is ten times that amount? Using equation (22) and a K_M value of 10 mg%, the enzyme would be 80% saturated at 40 mg%, and 98% saturated at the lethal blood ethanol concentration of 400 mg%[55]. This is an average change in the saturation of the enzyme of 0.5% for each 10 mg% change in the blood ethanol concentration, a very subtle change indeed. Because of this subtle change, Goldstein[56] remarked that

> "Thus, the kinetics would appear to be virtually zero-order (linear) throughout this range."

Widmark's ß Versus Michaelis-Menten's V_{max}

Figure 15 shows six concentration versus time graphs using computer generated data employing equation (16). The C_0 values used for the six sets of data are 400, 200, 100, 50, 25, and 10 mg%.

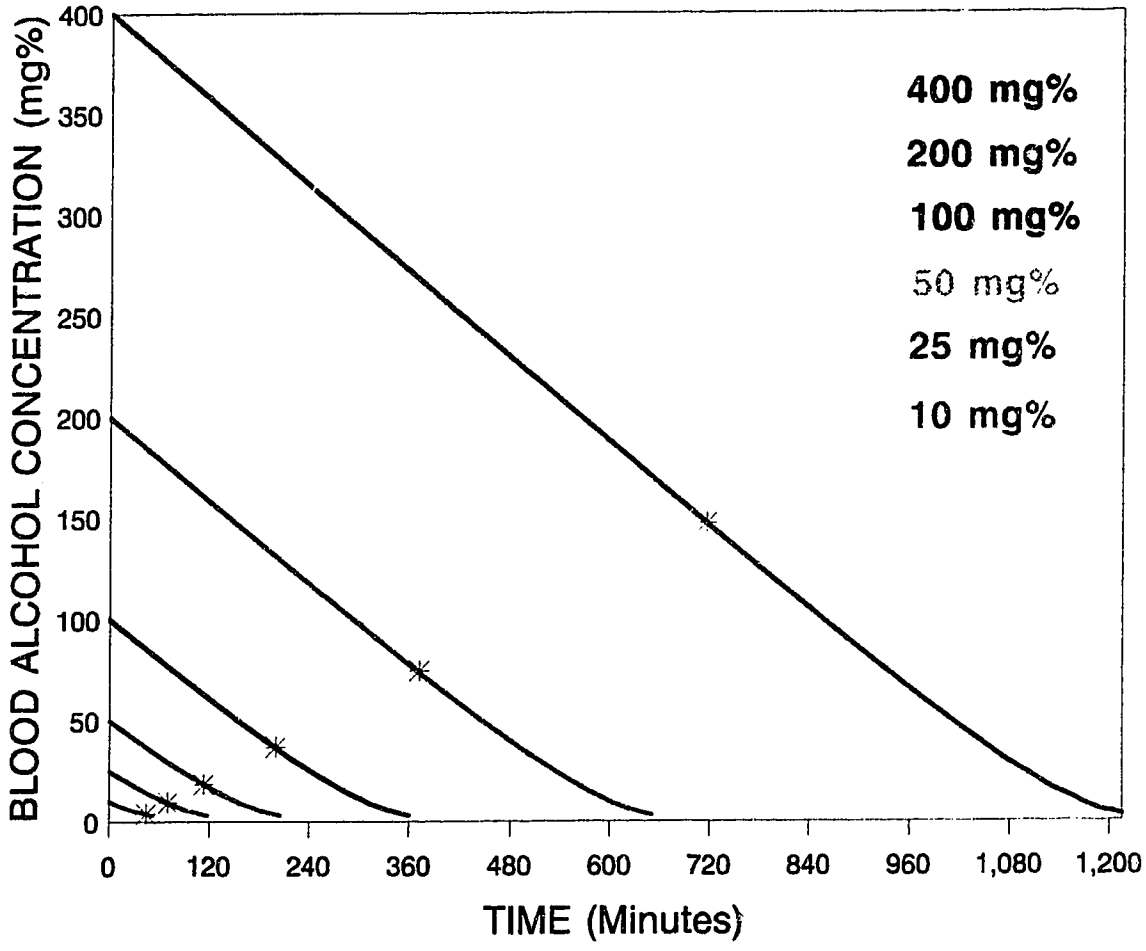


Figure 15. Six concentration versus time graphs using data computed from equation (16). V_{max} = 0.367 mg% per minute, K_M = 10 mg%, C_0 = 400, 200, 100, 50, 25, and 10 mg% respectively. The "*" indicates the t_{BRK} for each of the 6 graphs.

V_{max} and K_M were held constant at 0.367 mg% per minute (22 mg%/hour) and 10 mg% respectively for the six graphs. It can be seen from these graphs that the slope of the pseudolinear phase is the least for the C_0 = 10 mg% graph and the most steep for the C_0 = 400 mg% graph. Clearly the slope appears to be a function of C_0 as is the initial dose which is required to generate the C_0 alcohol concentration. Similarly, the ethanol concentration at time t_{BRK} is also a function of C_0 and therefore is also dose dependent. As well it can be seen that the six graphs are not superimposable, that each is distinct, their shape being a function of C_0, which in turn is a function of dose.

Figure 16 shows the relationship between the initial ethanol concentration, C_0, and the end of the pseudolinear phase, t_{BRK}. The t_{BRK}/C_0 value for each of the 6 graphs in Figure 14 was the highest for the C_0 = 10 mg% graph (t_{BRK}/C_0 = 4.45 minutes/mg%) and the lowest for the C_0 = 400 mg% graph (t_{BRK}/C_0 = 1.79 minutes/mg%). This further supports the contention that the six graphs in Figure 15 are not superimposable and that their individual shapes are a function of the initial dose given.

This whole concept of the initial dose establishing the subsequent shape and course of the concentration versus time graph raises a number of important questions. What biological mechanism is responsible for predetermining what the subsequent shape of a concentration versus time graph is to be based upon the initial amount of ethanol consumed? Why is it that the C_0 = 25 mg% graph in Figure 15 is not superimposable on the C_0 = 400 mg% graph from its concentration of 25 mg% onward? We are dealing with the same range of ethanol concentrations (25 to 0 mg%), and the same value for V_{max} and K_M.

Clearly, Michaelis-Menten kinetics are diametrically different from Widmark kinetics. Widmark asserts that elimination is independent of dose and the concurrent ethanol concentration; Michaelis-Menten proposes the opposite. The question remains, is the difference all that significant?

Table 9 is based upon the six concentration versus time graphs in Figure 15. Widmark's ß value for each of the six graphs was determined by linear regression analysis of the data points between the C_0 value and the concentration at the t_{BRK} point for each of the graphs. This value was then compared to the Michaelis-Menten value for V_{max} which remained a constant 0.367 mg% per minute for each of the 6 graphs.

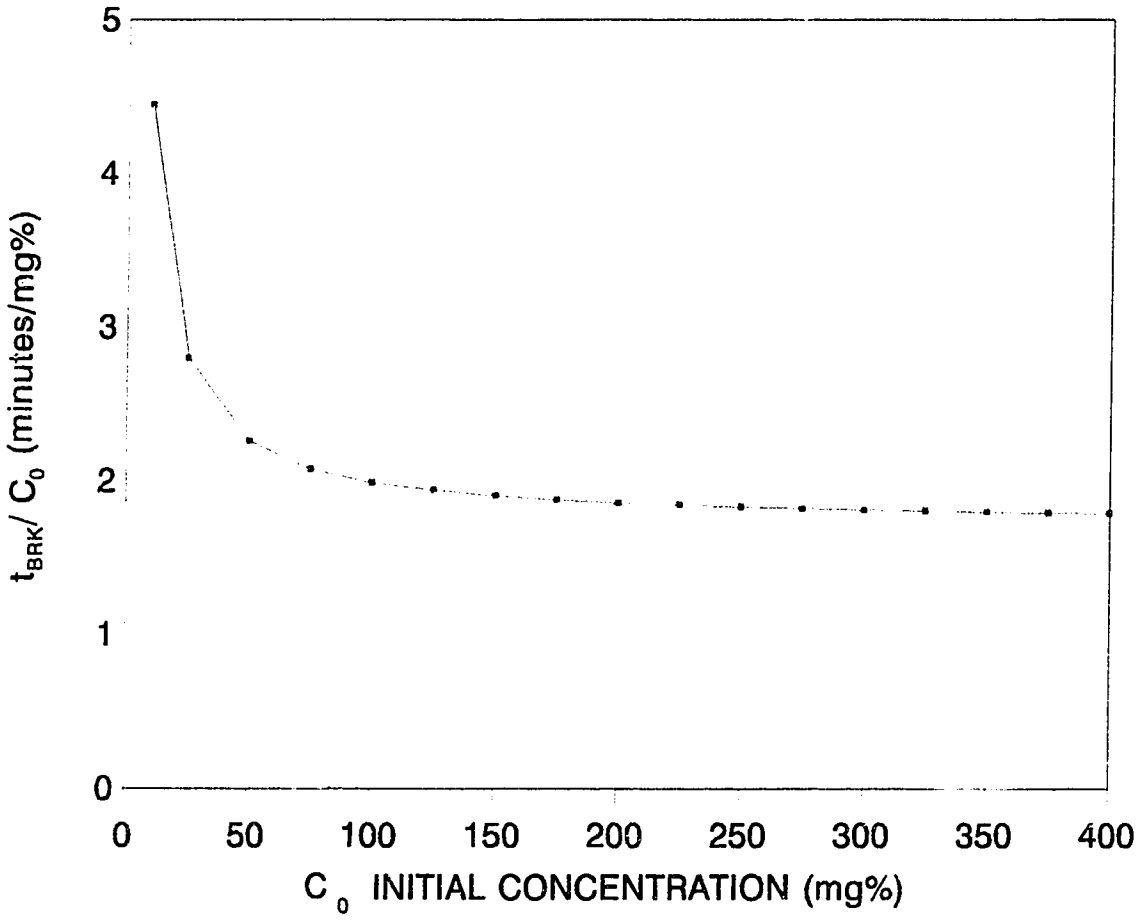


Figure 16. The relationship between the initial alcohol concentration and the end of the pseudolinear phase (t_{BRK}). When corrected for the initial ethanol concentration, the break point between the pseudolinear phase and the terminal curved phase is the greatest when C_0 = 10 mg%, and the least when C_0 = 400 mg%

between the two becomes minimal. What is interesting to note is that the value for Pearson's coefficient of determination (r^2) is very high for each of the six linear regressions; even when C_0 = 10 mg%. This indicates a strong linear relationship between concentration and time during the initial elimination phase even though the graph is actually curved.

Korsten, Matsuzaki, Feinman and Lieber - 1975

Questions have been raised in the past concerning the effect of chronic alcoholism on ethanol elimination rates. Korsten *et al*[57] studied the metabolic rate of alcohol by measuring the production of acetaldehyde in six chronically alcoholic males and five control male hospital patients. The age of these subjects ranged from 35 to 46 years with an average of 39 years. The alcoholic patients had been admitted to the hospital for treatment of delirium tremens and had reported drinking for a minimum of 10 years. In contrast, the control patients had been admitted for functional complaints not associated with the consumption of alcohol.

At the commencement of a testing session, each subject ate a standardized breakfast. This was followed by an intravenous infusion over the next three hours of a 15% (v/v) ethanol solution with 5% dextrose. Sequential blood samples were then collected through an indwelling catheter over the next 8 to 10 hours. Analysis of the blood samples was by head space gas chromatography.

In the alcoholic patients (Figure 17.), the acetaldehyde concentration remained fairly steady around a mean concentration of 42.7 ± 1.2 μ M over a wide range of ever decreasing blood ethanol concentrations. Once a mean ethanol concentration of 25 ± 2.0 mM was achieved, the concentration plateau ended and the concentration of acetaldehyde decreased progressively back to base- line values.

A similar phenomenon occurred with the control patients (Figure 18.) except that the acetaldehyde concentration initially maintained was significantly lower at 26.5 ± 1.5 μM and continued until a mean ethanol concentration of 22.4 ± 2.4 mM was achieved. This end point for the plateau was not significantly different between

Table 9.

Michaelis-Menten Versus Widmark Kinetics:

Comparison of V_{max} and ß

C_0 (mg%)	V_{max} (mg%/min)	ß (mg%/min)	V_{max}-ß/V_{max} (%)	Linear Regression r^2
10	0.367	0.146	-60.2	0.994
25	0.367	0.229	-37.6	0.998
50	0.367	0.282	-23.2	0.999
100	0.367	0.319	-13.1	0.999
200	0.367	0.341	-7.08	0.999
400	0.367	0.354	-3.54	0.999

Note: C_0 is the initial alcohol concentration. Pearson's r^2 is a measure of the goodness of fit for the linear regression. A perfect fit would provide an r^2 value equal to 1.000

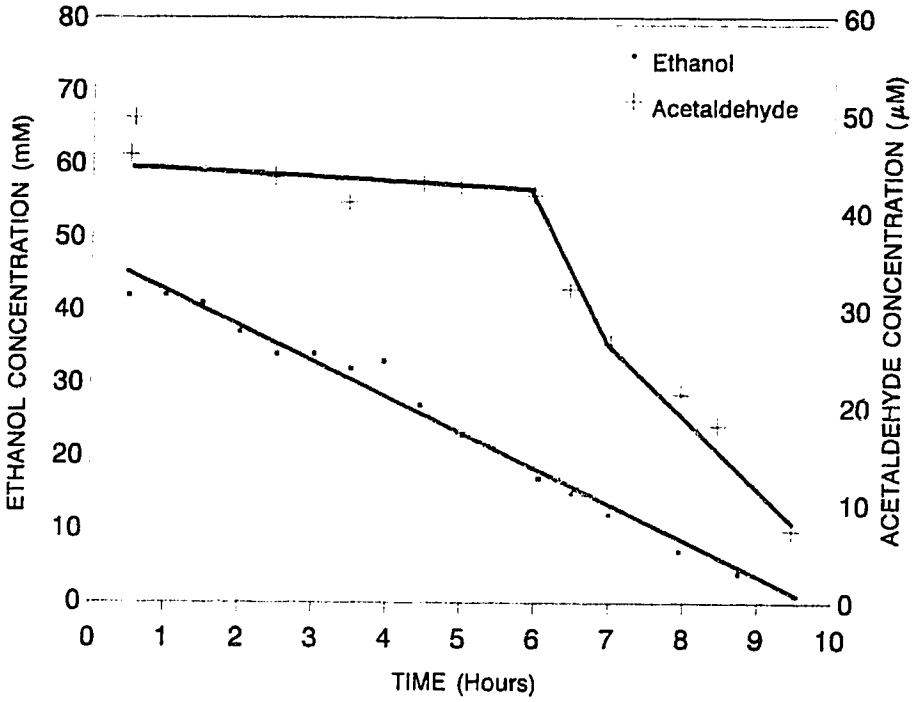


Figure 17. The acetaldehyde and alcohol concentrations in an alcoholic patient following the intravenous infusion of a 15% (v/v) alcohol solution.

Adapted from Korsten, Matsuzaki, Feinman and Lieber (1975) page 387

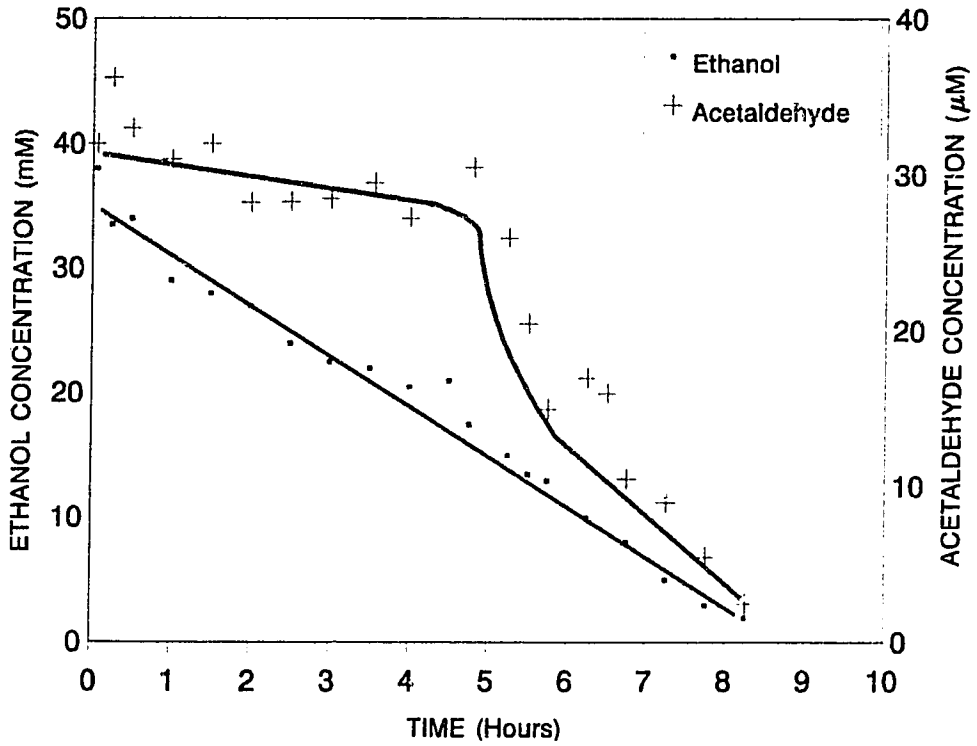


Figure 18. The alcohol and acetaldehyde concentrations in a nonalcoholic patient following the intravenous infusion of a 15% (v/v) alcohol solution.

Adapted from Korsten, Matsuzaki, Feinman and Lieber (1975) page 387

alcoholic and nonalcoholic groups. Similarily, there was not a statistically significant difference between the rate of ethanol disappearance from the blood in alcoholic subjects (23 ± 2 mg% per hour) and the non-alcoholic subjects (25 ± 2 mg% per hour).

Of the total amount of ethanol infused, approximately 5% was lost via the breath and urine. During the decline of the blood alcohol concentration, the K_M value for the alcoholic group was 2.3 ± 0.1 mM, which was not significantly different from the value for the controls of 2.5 ± 0.2 mM

The actual relationship between the concentration of acetaldehyde and alcohol at the high blood alcohol concentrations experienced by these subjects is as yet unknown. Clearly during the acetaldehyde concentration plateau the net effect of production and degradation of the acetaldehyde was zero. Therefore, following the plateau, either the production of acetaldehyde decreases, and/or its elimination from the blood is increased. Since the excretion rate of acetaldehyde is minimal, and an increase in metabolism is unlikely[58], it follows that the sudden and persistent decline in the acetaldehyde concentration following the plateau is due to a decreased production of acetaldehyde.

Korsten and his colleagues argued that since ADH is saturated while the acetaldehyde concentration is in its plateau, the increased production of acetaldehyde during the plateau phase must be by some other oxidative system with a K_M higher than that of the ADH. Since Lieber is one of the co-authors of this paper, it is not surprising that the MEOS previously discussed by Lieber *et al*[59] is implicated. Hence as the ethanol concentration decreases following the end of the acetaldehyde plateau, MEOS would become desaturated resulting in a diminishing production of acetaldehyde by MEOS, consistent with the post plateau decline in the concentration of acetaldehyde. The higher levels of acetaldehyde shown in the alcoholic subjects may be due to decreased catabolism of the acetaldehyde resulting from alcohol induced liver damage.

Bogusz, Pach and Stasko - 1977

These three researchers[60] compared the rate of alcohol elimination under conditions of high blood alcohol concentrations, and following a small dose of ethanol in the same person. The twenty-six male and three female subjects, ranging in age from 17 to 60 years, were initially undergoing treatment in a hospital for acute

ethanol poisoning. Their blood alcohol concentrations were measured upon admittance to the hospital, and 3, 6, and 12 hours following admission. The initial blood alcohol concentrations of these subjects ranged from as low as 134 mg% to 488 mg%. However, 21 of the subjects had blood alcohol concentrations in excess of 300 mg%.

For each subject, the rate of ethanol elimination was determined based upon the alcohol concentration and the time of the four blood samples taken. The hourly rates of elimination for these 29 subjects ranged from 6 mg% per hour to 36 mg% per hour with a mean of 21.9 ± 7.2 mg% per hour. The declines in the blood alcohol concentrations in these 29 patients, over the 12 hours observed, were noted not to be linear.

In an attempt to determine if there was a correlation between the coexisting blood alcohol concentration and the elimination rate, the rate was measured between the first sample and the second sample, *i.e.* upon admission and three hours later. This was then compared to the rate observed between the samples taken 6 and 12 hours post admission. A positive correlation coefficient r of 0.6426 was obtained which was significant at a level of $P = 0.001$. Therefore, at blood alcohol concentrations in the 300 mg% range, there is correlation between concentration and rate of elimination.

Once the patients had been treated to the point of recovery, 14 of the initial 29 patients were given oral doses of 0.667 ml/kg 95% by volume ethanol. Venous blood samples were taken one, two and three hours following the consumption of the alcohol. For each of the 14 subjects, the highest blood alcohol concentration was noted for the first sample taken one hour post consumption. The elimination rates for these 14 subjects were noted to be linear and averaged 14.6 ± 4.8. mg% per hour. This was significantly lower than the initial average rate during the 12 hours following admission ($t = 3.0544$; $P = 0.01$).

Bogusz *et al* give three reasons for the higher rates of elimination at the higher blood alcohol concentrations compared to the rates at the lower concentrations.

1. The proportion of ethanol excreted as unchanged alcohol, such as on the breath and in the urine, is greater relative to the rate of metabolism by ADH, which at these alcohol concentrations is saturated.

2. Widmark's ß is inversely related to the distribution of water throughout the body, *i.e.* Widmark's *r* factor[61]. It is possible that acute ethanol poisoning may modify the distribution of body water and thereby inversely affect the rate of elimination.
3. It is possible that, consistent with Lieber *et al*[62], MEOS may contribute to higher rates of ethanol oxidation at the higher concentrations.

Bosron and Li - 1977

Bosron and Li[63] provided yet another attempt to explain the elevated rates of elimination associated with high blood alcohol concentrations. They used affinity chromatography and starch gel electrophoresis to separate and purify to homogeneity a species of ADH which has a K_M value as much as 100 times greater than other molecular forms of ADH. During separation from the other isoenzymes of ADH on starch gel electrophoresis, this newly identified form of ADH distinguishes itself as having the slowest electrophoretic mobility, and therefore appears closest to the anode; hence the label, the anodic form of ADH.

The anodic form has a molecular weight of 42,000, similar to the other forms of ADH. It also shares similarities in its amino acid composition, contains 4 atoms of zinc per molecule of protein, has a similar K_M for NAD^+ and is inactive towards $NADP^+$. However its K_M for alcohol is 140 mM *at a* pH of 10, and 18 mM at a *p*H of 7.5, which is between 20 and 100 times greater than that reported for impure preparations of ADH. It is also distinctive in that it is inactive towards methanol.

Because of the higher K_M for this anodic form, it is argued that it may play a role in the elevation of elimination rates at blood alcohol concentrations which are consistent with enzyme saturation for the other isoenzymes of ADH.

Bruno, Iliadis, Treffot, Mariotti, Cano and Jullien - 1983

Bruno *et. al.*[64] were concerned with the forensic application of alcohol kinetics. They acknowledged that the use of zero order kinetics to describe alcohol elimination is inaccurate. At the same time they realized that the use of Michaelis-Menten kinetics is just too complex to use in the ordinary courtroom situation. They sought to develop a simple approach to describe the changes in

blood alcohol concentrations following oral administration of alcohol in two types of male subjects. The first group consisted of three nonalcoholic males; the second group was composed of five alcoholic males who had consumed at least 9 fluid ounces of alcohol a day for the past five years.

The alcohol was consumed following an overnight fast from solid foods. Whole blood samples were collected and analyzed by head-space gas chromatography. Once the maximum blood alcohol concentration had been reached, they noted that the decline in the concentration was tri-phasic. The first phase was characterized by a very rapid decline; the second phase appeared to be linear, with the final phase following 1st order kinetics. For the nonalcoholic subjects, the half life averaged 26.8 ± 4.0 minutes, which was significantly lower ($P < 0.05$) than for the alcoholic subjects for whom it was 31.4 ± 2.7 minutes. However, the $t_{\frac{1}{2}}$ values for each subject appeared to be independent of the dose of alcohol administered.

Bruno *et. al.* argued that the use of the half life of the terminal elimination phase is preferable to other commonly used methods for describing ethanol metabolism in the medico-legal context. Widmark's $ß_{60}$ is dose dependent and is therefore unreliable. The use of K_M and V_{max} are too complex to use in the courts because of the need for computers and a great number of experimental data points required to obtain the Michaelis-Menten parameters. They argued that the terminal phase is not related to experimental conditions but to the enzymatic activity. The major difficulty with this argument is that Bruno *et. al.* did not distinguish between elimination and metabolism. It is the net effect of absorption, distribution and elimination at a given point in time that the courts are interested in, not just that of metabolism.

Wilson, Erwin and McClearn - 1984

Wilson *et. al.* [65] provided an unique twist to the multiple dosing protocol. Forty six males were given initial doses of alcohol sufficient to raise their blood alcohol concentrations up to 100 mg%. Breath samples were collected and analyzed using an Intoximeter Gas Chromatograph Mark IV, beginning 10 minutes after the last consumption. During the first hour following dosing, breath samples were analyzed every five minutes; after that, every 30 minutes until a blood alcohol concentration of about 50 mg%

was achieved. A second dose of alcohol was then administered to bring the blood alcohol concentration back up to the 100 mg% level. Similar to the first session of breath testing, samples were collected every 5 minutes for the first hour following the second dose, and every 30 minutes for the next 2.5 hours. Ninety minutes following dosing was assumed to be the time required for complete absorption and distribution of the ethanol consumed. Following this 90 minute period, least-squares regression analysis was used to fit the best straight line to the descending data points. The goodness of fit for these fitted lines averaged 0.98.

The mean rate of elimination ($ß_{60}$) following the initial dose was 15.3 ± 3.4 mg% per hour. This was statistically lower than the average $ß_{60}$ of 17.8 ± 3.6 mg%/hour observed following the second dose ($P < .001$).

Wilson *et. al.* termed this enhanced rate of elimination following the second dose as acute metabolic tolerance to ethanol (AMTE). What must not be overlooked is that only 34 of the 46 males subjects exhibited an increased rate of elimination following the second dose. Of these 34, two subjects showed an increase of less that 1 mg%/hour. Eleven subjects showed a decrease in their $ß_{60}$ values and one subject showed no change at all. Therefore, 30% of the subjects did not exhibit AMTE. Of the remaining individual subjects, it is not known for which subjects the increase in $ß_{60}$ was statistically higher following the second dosing.

Winek and Murphy - 1984

Winek and Murphy were probably the first to compare zero order alcohol kinetics to first order alcohol kinetics[66] to see which theory better describes alcohol elimination. They concluded that when the consumption is known, 84% of the time zero order kinetics provided a more accurate prediction of blood alcohol concentration than did first order kinetics.

Twenty subjects, 10 classified as non drinkers and 10 as social drinkers were instructed to fast for at least three hours prior to the testing procedure. A non drinker was defined as one who consumed less than 6 ounces of ethanol per month. A social drinker was defined as one who consumed more. Each of the subjects was tested on two separate occasions (Trial 1 and Trial 2). The interval of time between testing sessions was not disclosed, nor the dose of alcohol administered, nor how the alcohol was administered. It is

known that 40 minutes was allotted to consume the ethanol. Breath testing, using the Breathalyzer® Model 1000, commenced 20 minutes following the end of the drinking period and every 15 minutes thereafter for the next 5 hours. The results were recorded as mg% and as the natural logarithm of mg%.

Paired blood alcohol concentration curves were constructed for each subject based upon the Breathalyzer® Model 1000 results versus the time of the breath sampling. For zero order kinetics, the plot was the alcohol concentration (mg%) versus the time of sampling. For first order kinetics, the plot was the natural log of the alcohol concentration (*ln* mg%) versus the time of sampling. The correlation coefficient for each type of graph indicated the degree of certainty of whether the plotted data points follow either zero order kinetics or first order kinetics (Table 10).

Winek and Murphy made two erroneous assumptions that are critical to the validity of their results. Firstly, they assumed that absorption of the consumed alcohol was complete when the peak alcohol concentration was reached. This is a common error in the alcohol literature. Prior to the peak blood alcohol concentration, the change in the concentration with time is the net effect of absorption of alcohol into the venous blood, the distribution of alcohol out of the blood into the surrounding watery tissues of the body, and the elimination of alcohol from the blood. Initially, of the three processes, absorption dominates and the concentration rises following first order kinetics. The peak alcohol concentration marks the transition between the dominant absorption phase and the domination of distribution and elimination. Absorption of alcohol will continue post peak but at an ever decreasing rate: a rate slower than the combined rate of loss of alcohol from the blood due to distribution and elimination.

The second error, again common in the literature, is assuming that following the peak blood alcohol concentration, the decrease in the concentration is due solely to elimination, and therefore, measuring the decline in the concentration during this period of time is a measure of the elimination rate. Widmark made it perfectly clear that this is not correct. To do so makes the assumed elimination rate an hybrid of the distribution rate and the elimination rate. Such measurements are an invalid reflection of the alcohol elimination rate. This may explain why Winek and Murphy obtained such variable correlation coefficients since peak alcohol concentrations and time to the peak are so variable (Table 10).

Table 10.

The Peak Blood Alcohol Concentrations

And the Correlation Coefficients

For Winek and Murphy's Twenty Subjects

Subject Number	Peak Blood Alcohol Concentrations (mg%)		Correlation Coefficients			
			Zero Order		First Order	
	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2
Social Drinkers						
1	127	98	-0.9767	-0.9668	-0.955	-0.98
2	172	68	-0.9262	-0.9621	-0.8988	-0.9525
3	156	87	-0.9444	-0.9893	-0.9533	-0.9608
4	192	25	-0.9214	-0.8697	-0.9053	-0.829
5	163	134	-0.9831	-0.9691	-0.9622	-0.9548
6	39	30	-0.9606	-0.9595	-0.8676	-0.9031
7	138	114	-0.9811	-0.9913	-7,857	-0.9741
8	89	84	-0.9888	-0.9629	-0.9505	-0.9214
9	80	54	-0.9219	-0.8845	-0.9584	-0.9285
10	75	53	-0.9734	-0.9837	-0.8913	-0.914
Non Drinkers						
1	118	71	-0.986	-0.8375	-0.9802	-0.8412
2	123	72	-0.9508	-0.9802	-0.9602	-0.9567
3	123	19	-0.9535	-0.5057	-0.9585	-0.476
4	157	101	-0.8184	-0.9496	-8,325	-0.9387
5	103	79	-0.9876	-0.9856	-0.9296	-0.9767
6	137	79	-0.9793	-0.9765	-0.9758	-0.9852
7	85	62	-0.9825	-0.9519	-0.9742	-0.958
8	66	39	-0.9399	-0.9614	-0.9446	-0.8848
9	71	57	-0.9734	-0.9846	-0.9226	-0.9495
10	129	106	-0.9732	-0.9858	-0.9799	-0.9759
Mean	117	72	-0.9561	-0.9329	-0.9293	-0.9131
± SD	40	30	0.039	0.109	0.052	0.112

Table 10 (con't)

Note: A statistical difference existed ($P<.001$) between the peak blood alcohol concentration achieved for Trial 1 and Trial 2. There was no significant difference ($P>.05$) between the zero order kinetic correlation coefficients for Trial 1 and Trial 2, and similarly for the first order correlation coefficients for Trial 1 and Trial 2. There was, however, a statistical significance ($P<.05$) between the zero order kinetic and the first order kinetic correlation coefficients for Trial 1. A statistical difference ($P<.01$) also existed between the zero order and the first order kinetic correlation coefficients for Trial 2. For both Trial 1 and Trial 2, the correlation coefficient was highest for zero order kinetics. There was, however, no significant difference ($P>.05$) between the correlation coefficients for zero order kinetics during Trial 1 and first order kinetics during Trial 2, nor for zero order kinetics during Trial 2 and first order kinetics during Trial 1.

Adapted from Winek and Murphy (1984) Page 164

Zero Order Kinetics

The rate of decline for zero order kinetics is equal to the change in the alcohol concentration with time.

$$(d[y]/dx) = -K \qquad (23)$$

Integration of this equation provides the following equation for a straight line

$$y = ax + b \qquad (24)$$

where y is the alcohol concentration at time x, a is the zero order rate constant, and b is the y intercept. The zero order rate constant is the slope of the graph, which is a negative slope.

The mean correlation coefficient for the 20 graphs of alcohol concentration (mg%) versus the time of sampling was 0.9496 ± 0.0783.

First Order Kinetics

The rate equation for first order kinetics is

$$(d[y]/dx) = -k[y] \qquad (25)$$

which integrated, gives an exponential equation for the relationship between alcohol concentration *[y]* and time x. where a is the first order rate constant and b is the y intercept.

$$[y] = be^{-ax} \qquad (26)$$

The natural logarithm of both sides of this equation results in a linear relationship between the alcohol concentration y and time x.

$$ln\ [y] = ln\, b - ax \qquad (27)$$

For the first order kinetic subject graphs, the mean correlation coefficient was 0.9270 ± 0.0841.

Table 11.

Peak Blood Alcohol Concentrations and Zero Order Elimination Rates For Winek and Murphy's Twenty Subjects

Subject Number	Peak Blood Alcohol Concentrations (mg%)		Zero Order Elimination Rate (mg%/hr)	
	Trial 1	Trial 2	Trial 1	Trial 2
Social Drinkers				
1	127	98	15	12
2	172	68	15	16
3	156	87	15	15
4	192	25	18	14
5	163	134	19	17
6	39	30	10	7
7	138	114	20	17
8	89	84	21	10
9	80	54	10	8
10	75	53	17	11
Non Drinker				
1	118	71	12	7
2	123	72	13	8
3	123	19	14	1
4	157	101	16	11
5	103	79	15	13
6	137	79	16	10
7	85	62	13	10
8	66	39	13	10
9	71	57	12	12
10	129	106	19	12
Mean	117	72	15	11
± SD	40	30	3	4

Table 11 (con't)

Note: A statistically significant difference exists between not only the peak blood alcohol concentratio[illegible]n Trial 1 to Tri[illegible]<.001), but also between the zero order elimination rates recoded for Tr[illegible]n Trial 2 (P<.001). For Trial 1 the rates vary from a minumum of [illegible] mg%/[illegible] to a maximum of 21 mg%/hour. For Trial 2, the range is significantly greater, [illegible] 1 mg%/hour to a maximum of 17 mg%/hour.

Adapted from Winek and Murphy (1984) Page 164

Zero Order Kinetics Versus First Order

Winek and Murphy concluded that because there was not a significant difference between the correlation coefficients for either zero order or first order kinetic graphs, and because the correlation coefficients for both types of graphs showed good linearity, this criterion could not be used to distinguish which was the better kinetic theory for describing alcohol elimination.

The major difference between zero order and first order kinetics is that for first order kinetics the rate constant is a function of the alcohol concentration at any given time. One of the parameters of first order kinetics is the half life value which is a function of the elimination rate. Winek and Murphy reasoned that if alcohol elimination follows first order kinetics, the half life values for each subject should be the same during Trial 1 and Trial 2 even though each trial produced different peak blood alcohol concentrations.

The Trial 1 and Trial 2 half life values were calculated for each subject. There was an average absolute difference between the Trial 1 half lives and those for Trial 2 of 96.3 minutes, or a mean difference of 33 ± 25%. Since this was a significant discrepancy between the half life values from Trial 1 to Trial 2 for individual subjects, Winek and Murphy concluded that alcohol elimination does not follow first order kinetics.

As a further test, Winek and Murphy compared the actual blood alcohol concentrations at 2 and 3 hours after the peak blood alcohol concentration was reached, to what was predicted using either the zero order kinetics or first order kinetics. Zero order kinetics provided predicted readings which were on average 11.2 ± 17.9% different from the actual two hour reading, and 17.9 ± 21.1% different from the three hour reading. When first order kinetics was used to predict the blood alcohol concentrations at the two hour post peak time, the average difference was 24.7 ± 21.0%. The difference between predicted and actual alcohol concentration at the three hour time was not given. From this test, Winek and Murphy concluded that since 84% of the time zero order kinetics made closer predictions of blood alcohol concentrations than did first order kinetics, that alcohol elimination is better described by zero order kinetics. What they neglected to conclude was that the zero order elimination rate, as determined by them, was significantly different from Trial 1 to Trial 2. (Table 11.)

Cole-Harding and Wilson - 1987

Much research has been conducted in the past concerning the effect that gender has on ethanol kinetics. Animal models have been used to show that females eliminate alcohol more rapidly than mature males [67,68]. However, when immature males were used, there was no significant difference between either immature or mature females. Higher levels of testosterone in the mature males is cited as the cause for the lower rates in the mature males [69].

In human studies, it has generally been agreed that consistent with Widmark's findings [1], women have proportionately more body fat than males and therefore the peak blood alcohol concentration is higher for females than for males of equal stature. Cole-Harding and Wilson [70] tested 63 women and 75 men to determine if there was a significant difference in elimination rates for the male and female subjects. Prior to administering the alcohol, each subject was weighed and interviewed concerning drinking and smoking habits, and for the women, the period of the menstrual cycle that they were in, and whether they were taking oral contraceptives. The dose of ethanol (1.0 ml) was administered four hours subsequent to a standardized breakfast. Topping up doses of alcohol were given to maintain a blood alcohol concentration over a three hour period. During this three hour period, a battery of psychomotor and cognitive tests was administered.

To establish the β_{60} for each subject, only those breath samples collected 60 minutes following the last topping up dose and down to a blood alcohol concentration of 20 mg% were used. It was therefore assumed that the distribution of the consumed alcohol was complete within 60 minutes of the last consumption. From Table 12 it can be seen that women eliminated alcohol more rapidly than the men and had a higher peak blood alcohol concentration than the men. However, this was not shown to be statistically significant. Contrary to earlier reports [71], those women taking oral contraceptives did not eliminate ethanol significantly differently from those women not taking oral contraceptives. Similarly, the phase of the menstrual cycle did not significantly alter β_{60}.

Nagoshi and Wilson - 1989

Nagoshi and Wilson[72] tested 39 subjects on two different days to determine, amongst other things, if there was a significant difference in an individual's alcohol kinetic parameters from one day

Table 12.

$ß_{60}$ Values and Peak Blood Alcohol Concentrations

For Male and Female Subjects

	$ß_{60}$ ± SD (mg%/hour)	Peak BAC ± SD (mg%)
Menstrual Phase:		
Low estrogen - low progesterone	20.7 ± 1.3	102.5 ± 4.0
High estrogen - low progesterone	19.64 ± 1.4	99.6 ± 5.5
High estrogen - high progesterone	20.91 ± 1.5	96.0 ± 3.4
No distinct phase	20.82 ± 1.1	99.6 ± 2.8
Taking Oral Contraceptives	21.83 ± 1.1	100.5 ± 4.1
All Women	20.77 ± 5.5[a]	99.7 ± 1.7
All Men	17.24 ± 4[a]	97.0 ± 1.8

Note: [a] The $ß_{60}$ for females is significantly higher than for the males ($P<.001$). For the women, the $ß_{60}$ values are not affected by the phase of the menstrual cycle, nor the taking of oral contraceptives. Although the peak blood alcohol concentration appears higher for the women than for the men, the difference is not statistically significant.

Adapted from Cole-Harding and Wilson (1987) page 382 - 383

to the next. From their testing, they concluded that there exists a near zero intrasubject repeatability in alcohol clearance rates.

The interval of time between the two testing dates ranged from 3 to 39 months (average = 20.7 ± 9.1 months). These paid subjects were part of a much larger sample known as the Colorado Alcohol Research on Twins and Adoptees (CARTA) recruited from the Denver, Colorado area. In addition to alcohol kinetic testing, the CARTA subjects were given a battery of cognitive, physiological and motor performance tests.

The average age of the 19 males and 19 females was 28.03 ± 4.46 years, with a minimum age of 21 years. The subjects were instructed to refrain from consuming food, alcohol, drugs, or beverages containing caffeine for the 12 hours preceding the testing. The subjects were to arrive at the testing facility at 8:00 a.m. and were given a "*small low-fat breakfast*".

The alcohol dosing and breath sample collection protocols used for the first day were different from the second day of testing. Following the breakfast on the first day of testing, neuropsychophysical testing was conducted until 12 noon at which time the first dose of alcohol (0.8 g/kg) was administered to the subjects. This ethanol was diluted with a non-carbonated, sugar free soft drink, and was consumed within 15 minutes. Topping up doses were administered one hour later and again two hours after the initial dose to maintain a blood alcohol concentration near 100 mg%. Subsequent to the final peak blood alcohol concentration being achieved, a lunch was provided. Breath samples were collected and analyzed using an Omicrom Systems Intoxylizer, and cross checked with a gas chromatographic Intoximeter Mark IV. Breath testing began 10 minutes following the initial dose and every 10 minutes thereafter for the next three hours. The breath testing interval was then increased to every 30 minutes until the alcohol level declined to 20 mg% at which time the testing concluded. The alcohol elimination rate was based upon the results of the breath tests taken 60 minutes following the last topping dose, and therefore, shortly after the lunch that was provided.

For the repeat testing day, the subjects again attended the testing facility at 8:00 a.m., were given the standard breakfast followed by a dose of alcohol (0.8 g/kg) shortly thereafter at 9:30 a.m. No topping up doses were provided. Ten minutes was allowed for absorption following the alcohol dosing. The first breath tests were conducted five minutes after the 10 minute absorption period, and

again 5 minutes later. Breath testing then continued at 10 minute intervals for the next two hours. This was followed by testing every 30 minutes until a 20 mg% alcohol concentration was achieved. The subjects were then dismissed. The elimination phase was assumed to commence 20 minutes after the peak alcohol concentration was achieved.

The alcohol kinetic parameters measured by Nagoshi and Wilson included time to peak blood alcohol concentration, the peak blood alcohol concentration, the alcohol clearance rate, and Widmark's *r* factor. Table 13 shows the difference in the mean values for these parameters from day one to day two of testing. Of the parameters tested, there was a statistical difference between the day one and the day two mean values for the time to peak alcohol concentration, the peak alcohol concentration, and the modified Widmark *r* factor. There was no statistical difference in the mean rate of clearance from day 1 to day 2.

Nagoshi and Wilson appear to make a number of silent assumptions in coming to their conclusion that individual alcohol kinetic parameters vary greatly from one day to the next. They appear to assume that

1. analyses of breath samples provide an accurate means of measuring the concomitant blood alcohol concentration,
2. reliable results can be achieved when breath samples are collected within 10 minutes of the last consumption of alcohol,
3. absorption of alcohol from the stomach is complete upon attainment of the peak blood alcohol concentration
4. in terms of the first day of testing, absorption and distribution of alcohol was complete within 60 minutes of the last topping up dose, and in the case of the second day of testing, within 20 minutes of the peak blood alcohol concentration being achieved
5. the clearance rate of alcohol is zero order
6. the presence of food has no effect on the rate at which alcohol is absorbed from the stomach and small intestines, nor the time to reach the peak blood alcohol concentration.

From a pharmacokinetic perspective, Nagoshi and Wilson's protocol is, to say the least, unorthodox. They have attempted to measure specific blood alcohol kinetic parameters without first controlling the variables. For example, the drinking and eating pattern of the first day of testing was not identical to the second day of testing. It is well known that the presence of food in the stomach inhibits the rate at which alcohol is absorbed, and diminishes the peak blood alcohol concentration ultimately achieved and increases

Table 13

Changes in the Alcohol Kinetic Parameters

As Measured by Nagoshi and Wilson

On Two Different Days in 19 Male and 19 Female Subjects

Parameter	Day 1	Day 2
Time to Peak Alcohol Concentration (minutes)	44.30 ± 19.80	53.62 ± 17.58
Peak Alcohol Concentration (mg%)	95.65 ± 14.07	80.62 ± 15.53
$ß_{60}$ Clearance Rate (mg%/hour)	-19.50 ± 4.78	-19.79 ± 5.16
Modified Widmark's *r* Factor	0.79 ± 0.12	0.87 ± 0.17

Note: For all parameters, except the clearance rate ($ß_{60}$), there was a statistical difference ($P<.05$) between the day 1 results and those for day 2.

the time to achieve it.[73,74,75] There is confusion in their protocol about when the post absorption/distribution phase (the α phase) ends and the elimination phase (the β phase) begins. What marked the beginning of the elimination phase for day one (60 minutes following the last administration of alcohol) and for day two (20 minutes following the peak blood alcohol concentration) is also uncertain. It is clear that what Nagoshi and Wilson were measuring was the change in their subject's breath alcohol concentrations and not the blood alcohol concentrations. Most breath testing devices used assume that there is the same amount of alcohol in one part of blood as there is in 2100 parts of deep lung (alveolar) breath.[76,77,78] However, since that convention was established, scientific means to measure the partitioning between breath and blood have become more accurate and precise. As a consequence, such a rigid adherence to the 2100 to 1 partition ratio is surely wrong. Table 15 shows the blood:breath partition ratios are variable, leading to the conclusion that blood alcohol concentrations determined from analyses of breath are probably not valid. Results of breath analyses should be reported as breath alcohol concentrations and not blood alcohol concentrations.[79] The failure of this first assumption is not fatal to Nagoshi and Wilson's conclusions as it can be argued that the post diffusion-equilibrium rate of decline of the blood alcohol concentration will parallel that of the breath. At worst, it shows a lack of understanding on their part of what their analytical results really mean.

The second assumption deals with the interval of time following consumption of alcohol which must elapse before reliable breath testing can commence. Generally 15 minutes must elapse following alcohol consumption before breath testing can commence. This interval allows for the dissipation of any mouth alcohol prior to testing. Residual mouth alcohol will combine with the alcohol from the lungs to provide falsely high breath alcohol readings. Nagoshi and Wilson began breath testing on day one just 10 minutes after alcohol was consumed. While this by itself is not fatal to their conclusions about clearance rates, it further shows a lack of understanding about breath testing technology and technique.

To conclude that absorption of alcohol is complete when the peak alcohol concentration is achieved is also most assuredly wrong. Prior to the peak concentration, the rate at which absorption is occurring exceeds the combined rate of distribution of alcohol from the blood and the rate of elimination of alcohol from the blood. As a consequence, the blood alcohol concentration undergoes a

Table 14.

Alcohol Clearance Rates

For Nagoshi and Wilson's Thirty Eight Subjects

Tested on Two Separate Days

Subject No.	Day 1 (mg%/hour)	Day 2 (mg%/hour)	% Difference
1	16.26	13.63	16.17
2	-	-19.91	-
3	16.13	-	-
4	22.27	23.95	-7.54
5	17.69	21.68	-22.55
6	17.29	15.49	10.41
7	15.07	16.38	-8.69
8	21.98	20.7	5.82
9	15.43	21.65	-40.31
10	17.21	19.87	-15.46
11	11.21	21.04	-87.69
12	13.39	16.94	-26.51
13	25.44	26.77	-5.23
14	22.79	4.8	78.94
15	23.62	25.22	-6.77
16	24.15	17.48	27.62
17	21.26	21.79	-2.49
18	17	21.08	-24
19	12.23	25.53	-108.7
20	21.1	12.69	39.86
21	17.73	14.85	16.24
22	16.24	28.76	-77.09
23	28.93	17.3	40.2
24	17.83	19.23	-7.85
25	19.68	11.15	-43.34
26	19.14	17.94	6.27

Table 14 (con't)

Subject No.	Day 1 (mg%/hour)	Day 2 (mg%/hour)	% Difference
27	-	30.25	-
28	28.8	28.64	0.56
29	25.1	22.85	8.96
30	16.71	21.31	-27.53
31	20.44	22.18	-8.51
32	-	25.22	-
33	18.67	18.17	2.67
34	27.9	14.92	46.52
35	24.11	24.45	-1.41
36	12.78	20.38	-59.47
37	22.49	-	-
38	13.99	24.19	-72.91
Average ± S.D.	19.49 ± 4.70	20.23 ± 5.30	-8.1 ± 40.29
Range	11.21 - 28.93	4.80 - 30.25	-108.75 - 78.94

Note: The % Difference = 100((Day 1 - Day 2)/Day 1) There is no statistical difference ($P > .05$) between the mean clearance rates for day one and day two.

Table 15.

Blood/Breath Alcohol Concentration

Partition Ratios

Researcher	Mean ± S.D.	Range	N
Jones[1]	2180 ± 189	1837 - 2863	21
Alobaidi[2]	2231 ± 279	1414 - 3133	10
Dubowski[3]	2280 ± 241	1706 - 3063	397
Jones[4]	2121 ± 161	1746 - 2574	10

Note: This Table shows the variablility of the blood-to-breath alcohol partition ratios. The researchers cited are: [1] Jones AW (1978) Variability of the blood:breath ratio in vivo. *J. Stud. Alc.***39**: 1931 - 1939. [2] Alobaidi TAA, Hill DW and Payne JP (1976) Significance of variations in blood:breath partition coefficients of alcohol. *Br. Med. J.* **2**: 1479 - 1481. [3] Dubowski KM and O'Neill B (1979) The blood/breath ratio of ethanol. *Clin Chem.* **25**: 1144. [4] Jones AW, Beylich KM, Bjørnegoe A, Ingum J, and Mørland J (1992) *Clin. Chem.* **38**: 743 - 747.

first-order rate of rise. At that point where the combined effects of distribution and elimination equal that of absorption, a peak concentration is reached. Following this peak, the blood alcohol concentration will enter into a decline due to the rate of absorption being less that the combined effects of distribution and elimination. Such absorption will continue until there is no longer any alcohol left in the stomach to be absorbed, except for that which re-establishes the equilibrium between the alcohol in the stomach contents and in the venous blood within the walls of the stomach and the small intestines. Because the peak blood alcohol concentration does not mark the end of absorption, and the times to achieve it are so variable, this parameter is of little if any value at all in kinetic studies.

The assumption that the elimination phase for their subjects began 60 minutes after the last consumption of alcohol during day one, and 20 minutes following the peak alcohol concentration during day two, is fatal to Nagoshi and Wilson's conclusions unless they can show that an equilibrium had been achieved in their subjects between the alcohol in the blood and the surrounding tissues. Because they failed to show the probability of this occurrence, their conclusions must be treated with suspicion.

It is interesting to note that Nagoshi and Wilson cite four articles written by either Wagner and/or Wilkinson. Both of these researchers firmly believe that *"zero-order kinetics are inappropriate for describing the elimination of alcohol in humans"*.[80] Nagoshi and Wilson appear to ignore Wagner and Wilkinson's detailed accounts of alcohol kinetics in favour of Widmark's zero-order kinetics, but neglect to cite Widmark in their paper. It is also interesting to note that the title of Nagoshi and Wilson's article refers to "*Human Alcohol Metabolism*", when in point of fact, they are measuring not the metabolism of alcohol but its elimination from the human body. It is not clear from their paper if they are cognizant of the difference.

Due to an apparent lack of understanding of alcohol kinetics and more specifically in failing to clearly define when the elimination phase of alcohol begins; confusion over the difference between the metabolism of alcohol and its elimination; a lack of understanding of breath testing technology and what breath alcohol concentrations results really mean, combined with a faulty protocol, one must view Nagoshi and Wilson's conclusions concerning the variability of alcohol kinetic parameters with the gravest of suspicion.

Passananti, Wolff and Vesell - 1990

One of the criticisms common to much of the research up to this point in time is conducting repeat testing under dissimilar conditions. Passananti *et. al.*[81] were careful to control the conditions under which their eight healthy male medical students were tested on four consecutive Saturdays. None of the eight subjects had any history of chronic exposure to chemicals known to alter drug metabolizing enzymes, nor was there any history of smoking, regular consumption of drugs or alcoholic beverages. Not only was the day of the week held constant, but so was the dose of ethanol to be consumed (1 ml/kg 95% ethanol in 250 ml ice-cold water); consumption always began at 9:00 o'clock a.m. following at least 12 hours of fasting from solid foods and lasted for a specified period of 15 minutes; blood sample collection began at 11:00 p.m. and every half hour thereafter until 2:00 p.m. with a total of seven blood samples collected. Each sample was collected directly into a sterile vacutainer containing sodium fluoride and potassium oxalate.

Blood alcohol standards were prepared by adding known amounts of ethanol to whole human blood that contained sodium fluoride and potassium oxalate in the same concentrations as was known to be in the samples of the subjects' blood. Analysis of both the subjects' blood and the standards was by head space gas chromatography.

Four blood alcohol concentration profiles were constructed for each of the eight subjects. Linear regression analysis of each of these profiles resulted in an estimation of C_0 and k_0. From Table 16 it is apparent that while the value for C_0 did not change appreciably for each individual subject, there was variability between subjects. In fact, while the average intrasubject coefficient of variation was 0.067 with a range of 0.050 to 0.105, the mean intersubject coefficient of variation was 0.10 with a range of 0.072 to 0.120, or on average just over 49% higher than for the intrasubject variation.

Table 17 similarly shows that the k_0 values were quite predictable for each individual subject; however, there was significant variation between individuals tested in this study.

Again, while the mean intrasubject coefficient of variation was 0.095 with a range of 0.034 to .122, the mean intersubject coefficient of variation was about just over 38% higher, at 0.131 with a range of 0.095 to 0.168.

Table 16.

Repeat C_0(mg%) Values for Eight Male Subjects

Tested on Four Consecutive Saturdays

Subject	Week 1	Week 2	Week 3	Week 4	Mean ± SD	Coefficient of Variation (%)
1	80	100	100	100	95 ± 10	10.5
2	90	90	90	100	92.5 ± 5	5.4
3	100	90	100	90	95 ± 6	6.32
4	100	90	90	90	92.5 ± 5	5.4
5	100	100	90	90	95 ± 6	6.32
6	100	90	100	90	95 ± 6	6.31
7	90	90	100	80	90 ± 8	8.89
8	120	120	110	110	115 ± 6	5.22
Mean ± SD	97.5 ± 12	96.3 ± 11	97.5 ± 7	93.8 ± 9		
C of V (%)	12.3	11.4	7.17	0.098		

Table 17.

Repeat k_0 (mg%/hour) Values for Eight Male Subjects Tested on Four Consecutive Saturdays

Subject	Week 1	Week 2	Week 3	Week 4	Mean ± SD	Coefficient of Variation (%)
1	10	13	13	13	12.2 ± 2	16.4
2	9	11	11	12	10.8 ± 1	9.25
3	13	11	12	13	12.2 ± 1	8.2
4	13	10	11	12	11.5 ± 1	8.7
5	14	14	13	12	13.2 ± 1	7.58
6	13	12	15	12	13 ± 1	7.69
7	11	11	13	10	11.2 ± 1	8.93
8	15	15	15	10	14.8 ± 1	6.76
Mean ± SD	12.2 ± 2	12.1 ± 2	12.9 ± 2	12.2 ± 1		
C of V (%)	16.4	16.5	15.5	8.2		

Using one-way ANOVA with repeat measurements for both C_0 and k_0, Passananti *et al* showed that with the four separate administrations of ethanol given to the eight subjects, each subject remained consistent from one test to another, but each of the eight subjects differed from one another ($P<0.01$).

Summary

The balance of the scientific literature dealing with alcohol kinetics generally attempts to consolidate the previous research into an acceptable explanation of alcohol kinetics [82,83]. This process is made more difficult when one attempts to place such conclusions in a forensic context. In Canadian courts, Widmark's hypothesis[1] (1932) is almost exclusively used as an explanation for the rate of alcohol elimination in humans. It is a concept which can be readily grasped by lawyers, judges and jurors who have had little or no previous scientific training. Unfortunately, it is most certainly wrong, because Widmark's ß is a function of dose. On the other hand, there is clearly a large body of scientific evidence which asserts that Michaelis-Menten[7,32,41,84] kinetics describes the termporal changes in human blood alcohol concentrations better. Unfortunately, in the forensic context, such an approach requires computer assistance to track the nonlinear decline in blood alcohol concentrations. As well, there is a very practical problem with such an approach. Michaelis-Menten kinetics deal solely with metabolic oxidation of ethanol. They do not take into account the amount of alcohol lost due to excretion on the breath, urine, feces, sweat and tears, as does Widmark's hypothesis. Therefore, by itself, Michaelis-Menten treatment is impractical, and somewhat misleading to use in the courtroom situation to describe the changes in a subject's blood alcohol concentration with time.

The legal controversy to this is the question of whether there is any mischief caused by using either Widmark linear kinetics or Michaelis-Menten nonlinear kinetics to describe to a court of law the changes in a person's blood ethanol concentration. If one quotes an elimination rate for a blood alcohol concentration in the 25 to 50 mg% range, will the rate at higher blood alcohol concentrations be so significantly different that it will pervert the course of justice?

Forrest [85] argued that it does not. He looked at the computer generated elimination rates for blood alcohol concentrations between 25 and 200 mg% using Lewis's values of K_M and V_{max}[86]. He

noted that for a person of normal metabolism, the difference in the elimination rate between a blood alcohol concentration of 50 mg% and 200 mg% was only 2.6 mg%/hour. He therefore concluded that a difference of only 2.6 mg%/hour is insignificant when compared to the normal analytical error encountered when analyzing a blood sample.

Can the same be asked of an individual's rate of ethanol elimination from one day to the next? Is there a significant difference? Vesell *et. al.* [38] and Passananti *et. al.* [70] would argue that there is not a significant difference from one day to the next. While Kopun and Propping[87] may have disputed the findings that Vesell *et al.* [42] gave concerning the influence of genetic involvement in one's elimination rate, they were unable to show that individual elimination rates vary from day to day based upon their twin studies. In their 1987 paper. Wilson and Nagoshi[88] claimed that the test-retest correlation coefficient for alcohol clearance was found to be near zero. Unfortunately, for reasons cited earlier, their results cannot be viewed as pharmcokinetically valid. They neglected to advise how the blood alcohol concentrations were measured. Additionally, their protocol did not seem to distinguish between the kinetics of alcohol metabolism and the kinetics of excretion. The third major problem with their findings, which was repeated in a subsequent paper[89], was that it was not clear at what point in the blood alcohol concentration curve they started to measure elimination. In addition, they were inconsistent with the conditions of the testing protocol. The range of elimination rates ($ß_{60}$) for the first day of testing was from as low as 11.21 mg%/hour to 28.93 mg% / hour with a mean of 19.50 ± 4.775 mg%/hour. There was no statistical significance between the mean elimination rate of the first day of testing and the second ($p = 0.931$) which had a wider range of elimination rates (4.8 mg% /hour to 17.5 mg%/hour) and a mean rate of 19.79 ± 5.163 mg%/hour. What is interesting to note is that for one subject, the apparent rate fell from 22.79 mg%/hour to 4.8 mg%/hour by the second day of testing. Unfortunately, the measurement of goodness of fit for these elimination rates was not provided.

There are several factors which have been attributed to altering the rate of elimination in humans. Clearly, if the alcohol is consumed on an empty stomach, the alcohol concentration in the hepatic venous blood will be significantly higher than in the periphery. This disproportionately higher ethanol concentration in the liver will drive the elimination rate closer to V_{max} than the blood alcohol

concentration in the periphery would indicate. Therefore, it would appear that the condition of the stomach in terms of its contents would have an effect on elimination rates.

A second factor appears to be smoking. Kopun and Popping [87] showed that in those subjects who drank more than 76 millilitres of ethanol per day and smoked more than 25 cigarettes per day, the rate of elimination was increased by 45% above the rate of those who neither drank nor smoked. For those non-smokers who drank more than 76 millilitres per day, their average rate only increased by 20% above the elimination rate of those classified as non-drinkers and non-smokers.

The role that hormones play in human elimination is still a question requiring answers. It seems quite possible that testosterone in males decreases liver ADH activity [90], and quite possibly the impact is greater than the effect of oestrogen on female elimination rates [91]. However, whether women eliminate alcohol more rapidly than men has still not been resolved. Some would say that there is a significant difference in the rates between men and women [92,93]; others challenge that, saying that there is no significant difference in the rate [94].

While oestrogen-containing oral contraceptives have been known to alter the metabolism of some drugs, it has yet to be determined if that effect extends to the elimination of ethanol in women. As Holford [95] observed, the difference in elimination rates observed by Jones and Jones[96] may be explainable in part to a difference in the volume of distribution between the women taking oral contraceptives and those not.

2. Experimental Procedure and Results

Scope and Objectives

This research attempted to establish the variability of individual pharmacokinetic parameters for the elimination of ethanol in humans. The questions to be answered were:

1. What is the range of β_{60} for a normal population of male and female drinking drivers?
2. What is the mean β_{60} and standard deviation for the males, and is it different from the females?
3. What is the individual variability of β_{60} for the males, and if possible, for the females?

The study will be divided into two sections. The first section will analyze previously established elimination rates for about 735 subjects (Group I) who have been charged with drinking and driving offences and were tested over the past fourteen years. These subjects were tested on one occasion only. Least squares fit linear regression analysis was employed to determine their individual β_{60} values. A range, mean and standard deviation of these β_{60} values will be determined.

The second section of this thesis will report data from 29 subjects (Group II) who were tested for their elimination rates on two successive days. Following the consumption of ethanol by each subject, multiple breath samples were collected, analyzed by an infrared spectrophotometer (Intoximeter 3000) and the results plotted to produce individual alcohol concentration profiles.

The technique of least squares fit was used to fit a linear regression line to the breath alcohol concentrations in excess of 20 mg/210 ml of breath which occur during the second (post diffusion equilibrium) period of the alcohol concentration profile. The range and mean β_{60} were determined for these subjects, as well as the individual variability for each of these parameters.

The variability of the individual elimination parameters (from Group II subjects) was then compared to the range of parameters from the Group I subjects to see if there was a significant difference between the two groups.

Details of the Method

Subject Selection

Two groups of subjects were chosen from the general public who had been charged with a drinking and driving offence. The first group (Group I) comprised 735 individuals who had been tested over the last 15 years. This represents a sample size of drinking drivers tested for elimination kinetics which is larger by far than has ever been published in the scientific journals. These subjects have been tested on one occasion and constitute a data base of individual elimination rates resulting from peak breath ethanol concentrations of no more than 90 mg/210 L. of breath.

The second group of 29 subjects (Group II) were recruited from the Group I subjects who volunteered for retesting to measure the intrasubject variability of elimination parameters. The interval between the time of the first testing and testing for the second time was variable.

Subject Preparation for Testing

All subjects were instructed (see Appendix A.) not to consume any solid food for the five hours prior to attending the laboratory. They must have had a zero breath ethanol concentration upon arrival for the testing. In the interests of safety, no subjects were taking any medication which, combined with the ethanol consumed in the lab, would place their health and safety at risk. If the subject had a history of alcoholism or diabetes, written permission from their physician to perform the testing was required prior to the testing date.

All subjects were advised in writing what the testing entailed prior to the testing date, as well as orally immediately prior to the testing in the laboratory (see attachment). These procedures were approved by the Research Ethics Board of the Faculty of Medicine, protocol 833, dated February 1, 1993.

Testing

The 735 subjects comprising Group I received written instructions prior to attendance at the laboratory. They were instructed to refrain from consuming any ethanol for at least 24 hours prior to their attendance at the laboratory. In addition, they were to fast for

five hours before arriving at the laboratory. Upon arrival, each subject was weighed, given a short interview (see Appendix B.) and then given a breath analysis to ensure a zero residual blood alcohol concentration. Each subject was then given a drink of 95% (v/v) ethanol diluted with orange juice. The time that consumption commenced was recorded, as well as the time that the drinking ceased. A fifteen minute period of time elapsed following the last drink to minimize the possible effect of mouth alcohol on subsequent breath analyses. Breath testing continued for the next four to five hours until the blood alcohol concentration decreased to about 20 mg/210 L of breath.

Subjects tested between March 1984 to September 1987 were tested using the Gas Chromatographic Intoximeter Mark IV (Intoximeters Inc., St. Louis MO.). Those tested subsequent to April, 1989 were tested with the Intoximeter 3000 (Intoximeters Inc., St. Louis, MO.), an infrared spectrophotometer (Appendix D). The result of each analysis was plotted on graph paper against the time that the sample was collected.

Linear regression analysis was used to fit the best elimination line to the data occurring in the post diffusion equilibrium phase of the ethanol concentration versus time graph. The elimination rate, the value of C_0 and Widmark's *r* factor as well as Pearson's *r* (correlation coefficient) were calculated for each subject.

The Group II subjects were tested on two different days. The interval between the two testing days was variable. The same rules as for Group I concerning abstinence from ethanol and fasting also applied to this group. The ethanol consumed by this group was administered as 95% (v/v) ethanol diluted with orange juice. A period of 15 minutes elapsed from the time that consumption ceased and the taking of the first breath sample for analysis. Each breath sample was collected and analyzed by an Intoximeter 3000 infrared breath alcohol analyzer. Breath testing continued until the subject's breath alcohol concentration had declined to between 20 and 30 mg/210 L. The result of each analysis and the time (minutes elapsing since commencement of drinking) that the sample was collected was then plotted on a graph to produce a breath ethanol curve.

Analysis of Data

For each subject's breath ethanol curve, visual inspection was used to estimate where the elimination phase (ß phase) of the curve begins, that is, the fully post absorptive period following the attainment of diffusion equilibrium. Least squares fit was used to fit a linear regression line to the data points comprising the ß phase. Pearson's correlation coefficient *(r)* was used to measure the corresponding correlation coefficient for each of the regression lines fitted. The hourly rate ($ß_{60}$) equaled the slope of that line multiplied by 60 minutes.

The relationship between two variables such as the time to reach diffusion equilibrium and the time to consume the ethanol was tested using correlation techniques such as Pearson's *r* and point biserial correlation coefficient (r_{PB}).

The two tailed *t*-test for equality of means within independent samples was used to determine if there was a significant difference in kinetic parameters between male and female subjects. The two tailed *t*-test for paired samples was used to determine if there was a significant difference in the kinetic parameters for Group II subjects on different days. The probability of the two populations' means being equal was given in brackets as ($P =$). A *P* value less than 0.05 indicates a rejection of the null hypothesis that the two sample (or population) means are equal and therefore that a statistically significant difference exists between the sample's kinetics. Similarly, when a test for correlation is used, a *P* value less than 0.05 indicates a rejection of the null hypothesis that the correlation coefficient is zero. This would therefore indicate that there is a correlation between the two variables.

The range, mean, and standard deviation of the elimination parameters such as $ß_{60}$, time to diffusion equilibrium, C_0 and Widmark's *r* factor were calculated for the Group II data. These data were then compared to Group I data to see if there was a statistical significance between the two groups of subjects. Any day to day variations between the individual values of $ß_{60}$, C_0, Widmark's *r* factor and time to diffusion equilibrium were tested to see if they were statistically significant. Finally, the variations in individual elimination parameters were tested to see if they were statistically different from the range for Group I.

Table 18.

Description of Group I Subjects

By Age, Height and Weight.

Subjects	Mean ± SD	Range	N
Females			59
Age	31 ± 9	17 - 55	
Height (m)	1.65 ± .07	1.52 - 1.80	
Weight (kg)	66.39 ± 12.75	49.9 - 113.4	
Males			676
Age	34 ± 11	16 - 72	
Height (m)	1.78 ± 0.7	1.52 - 2.03	
Weight (kg)	79.82 ± 13.14	52.160 - 140.62	

Results And Discussion for Group I Subjects

Characteristics of Sample

Table 18 shows the makeup of the 735 Group I subjects tested. This represents a sample size larger than ever published, of drinking drivers who have been tested for their elimination rates. Of the 735 subjects, 59 were women ranging in age from 17 to 55 years with a mean height of 5'5" and a mean weight of 146 pounds. The 676 males ranged in age from 16 to 72 years old with a mean height of 5'10'and a mean weight of 176 pounds. Figure 19 shows that the distribution of age for the Group I subjects. The mean age for males was 34 years, and for the females the mean age was 31 years. The distribution of age is positively skewed for both males and females.

Dose and Period of Consumption of Ethanol

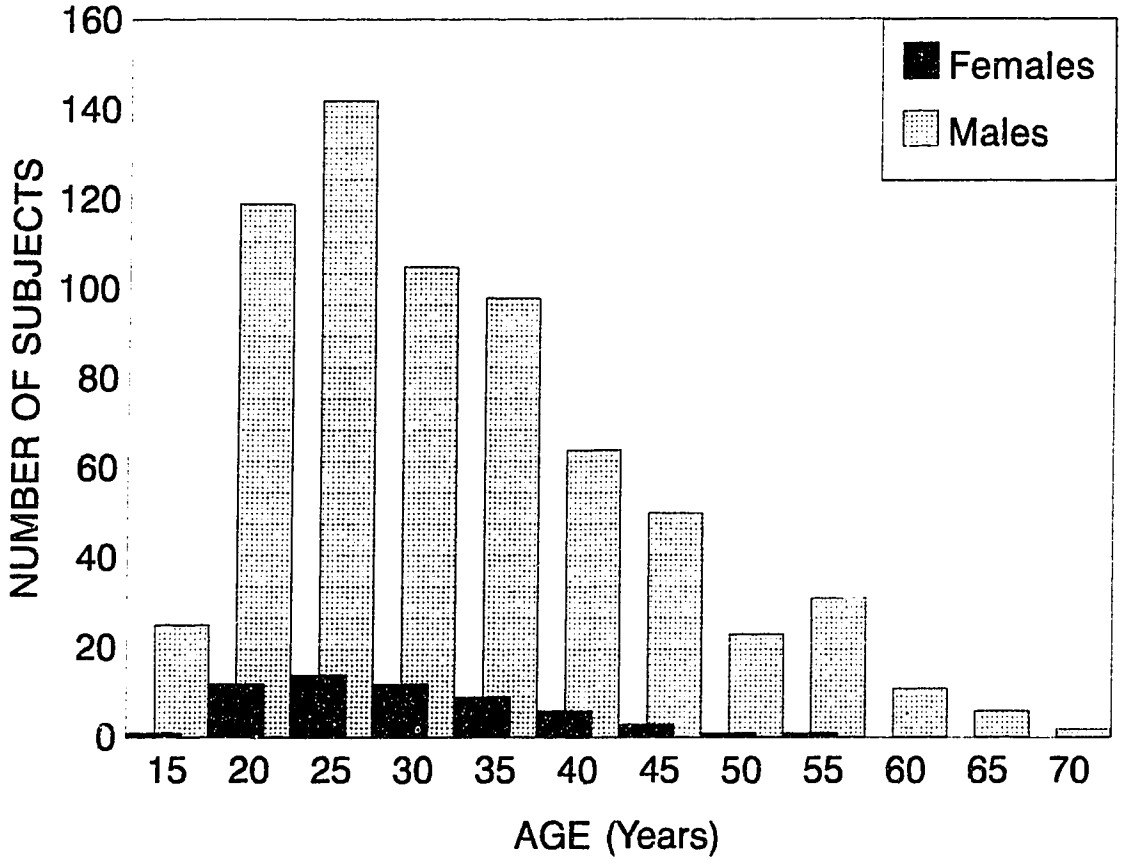
The dose of alcohol administered to these subjects has varied over the last 15 years. In general, it has been a volume of alcohol which would theoretically raise the subject's breath alcohol concentration to between 85 and 100 mg/210 L. The mean dose for the males was 0.75546 ± 0.10 ml/kg of 95 % by volume ethanol (0.454 - 1.064 ml/kg) and for the females it was 0.65443 ± 0.09 ml/kg (0.464 - 0.922 ml/kg). On average, consumption lasted for about 21 ± 10.3 minutes (1 - 91 minutes). To compensate for the effects of any residual alcohol left in the mouth following consumption, at least 15 minutes was allowed to elapse before the first breath sample was collected and analyzed.

Plotting Breath Alcohol Concentration Curves

Immediately following the analysis of each breath sample, the result was plotted on Gaussian graph paper. Figure 20 shows a typical plot of ethanol concentration versus time. The time for the collection of each sample is measured in minutes from the beginning of drinking.

Linear Regression Analysis of Elimination Phase

Choosing the beginning of the elimination phase of the ethanol concentration curve is a two step process. Following the peak in the ethanol concentration, ethanol continues to rapidly diffuse from the



MEAN Males = 34 +/- 11.27; Females = 31 +/ 8.54
SKEWNESS Males = .83; Females = .60

Figure 19. Distribution of age in Group I sample of 735 drinking drivers.

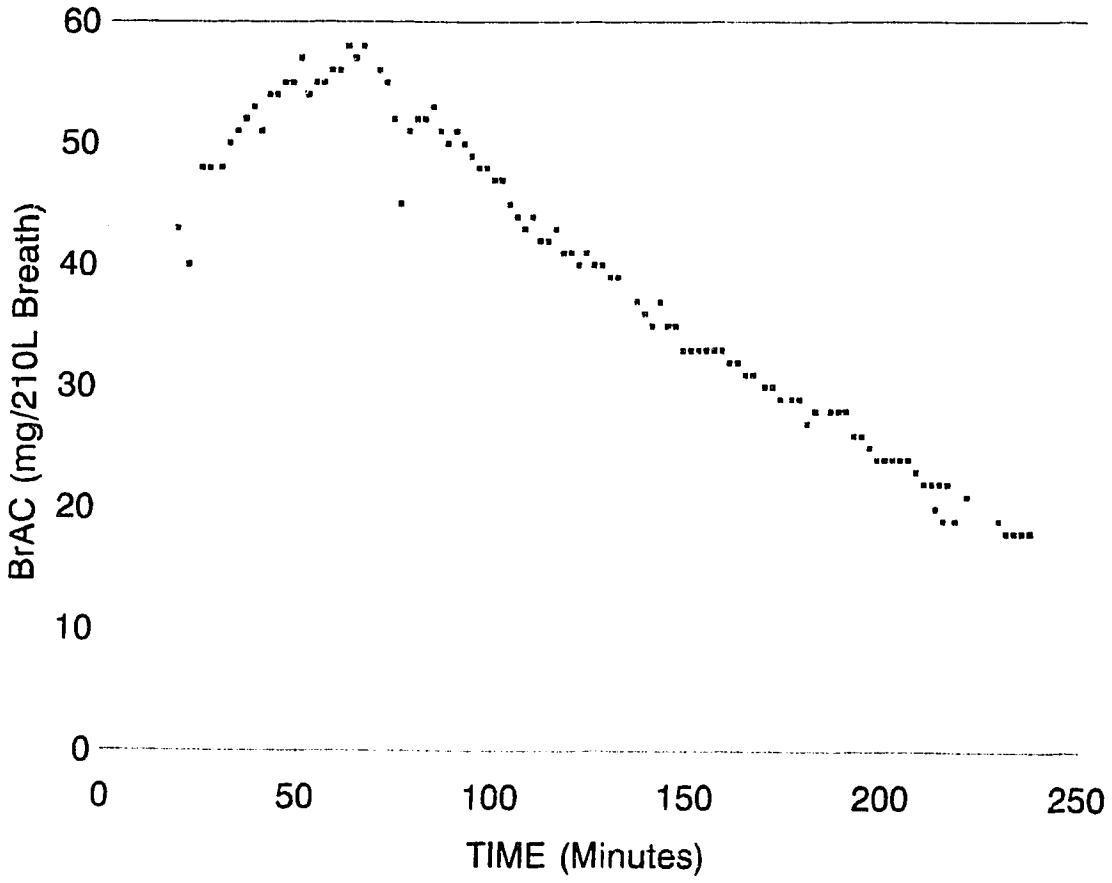
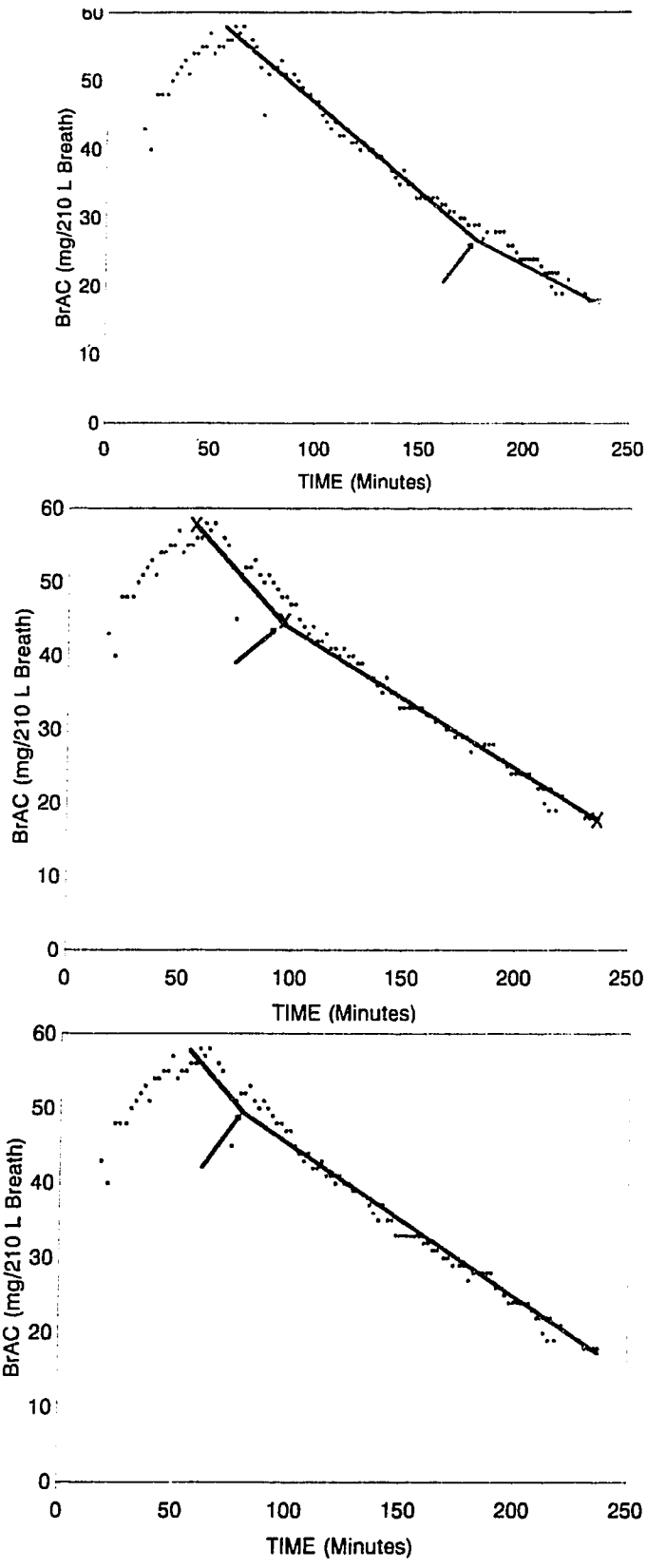


Figure 20. Breath ethanol concentration versus time . Subject Number 612 is a 6 foot tall, 36 year old male, weighing 185 pounds, who consumed 0.483 grams of ethanol per kilogram body weight within 12 minutes. The time to reach diffusion equilibrium was 102 minutes from the commencement of drinking. $ß_{60}$ = - 12.1 mg/210 L per hour, r = - 0.99534, C_0 = 64.7 mg/210 L, Widmark's r factor = 0.75


60
50
40
30
20
10
0
BrAC (mg/210 L Breath)
0
50
100
150
200
250
TIME (Minutes)
60
50
40
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20
10
0
BrAC (mg/210 L Breath)
0
50
100
150
200
250
TIME (Minutes)
60
50
40
30
20
10
0
BrAC (mg/210 L Breath)
0
50
100
150
200
250
TIME (Minutes)

Figure 21. Identifying and fitting the best line to the elimination phase of subject 612. For each of the three panels, a green arrow points to the presumed point of diffusion equilibrium. The top alcohol concentration chart shows a presumed point of diffusion equilibrium at 182 minutes. Clearly the blue segment of the line is too high above the data points associated with that phase. By moving the mid point backwards in time, the blue segment of the line becomes more aligned with the data points below it while pulling the red elimination phase segment up the alcohol concentration curve. The middle panel shows the best fitting line chosen for this subject. The blue segment lies fairly close to the data points associated with the post absorption distribution/elimination phase, and the second segment falls well within the data points for the elimination phase. By pushing the DE point back too far, as in the bottom panel, not only is there a loss of correlation between time and alcohol concentration during the lower phase, but the line is raised above the corresponding data points (Table 19).

Table 19.

Identifying the Point of Diffusion Equilibrium

And the Beginning of the Elimination Phase

t_1 (minutes)	t_2(minutes)	ß (mg/210 L minute^{-1})	Pearson's *r*
119	237	-0.197	-0.988
111	237	-0.199	-0.989
103	237	-0.201	-0.991
101	237	-0.204	-0.99
95	237	-0.207	-0.989
88	237	-0.212	-0.986

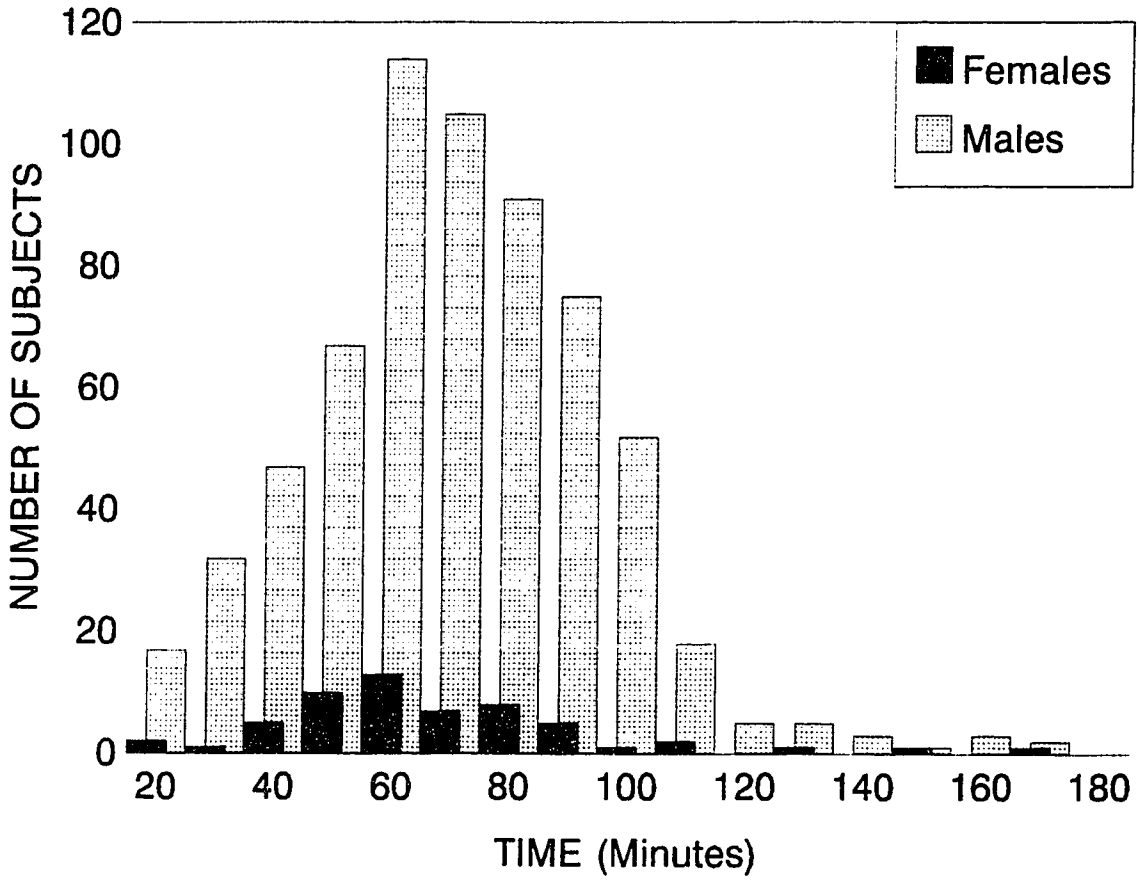
Note: This Table refers to the panels shown in Figure 21. As the predicted point of diffusion equilibrium (DE) is moved back in time from 119 minutes to 111 minutes and again to 103 minutes, there is a gradual increase in the ß values and an increase in the correlation coefficient (Pearson's *r*). Moving DE beyond 103 minutes produces a more dramatic increase in the ß values but a deterioration in the correlation coefficients. For this particular subject, the point of DE is at 103 minutes from the onset of consumption

blood into the surrounding tissues. This, combined with the elimination of ethanol from the blood, causes a net rapid decline in the graph. As the point for diffusion equilibrium approaches, this decline tends to level off somewhat. It is sometimes possible to see the change in the slope of the graph from the rapid diffusion/elimination phase to just the elimination phase simply by looking obliquely at the graph. The area of the graph where this change in slope takes place marks the beginning of the elimination phase.

The first step is to begin to fit a straight line to the elimination phase data points using least squares fit. A linear regression analysis computer program was written which makes use of three points on the alcohol concentration profile. The first point is the highest data point on the graph. The next, or the middle point, is the estimated point of DE. The third is the last data point on the graph. The elimination phase chosen will therefore be through those data points which are between the DE point and the last data point.

The initial attempts to fit the line should start with a predicted DE point which is well within the elimination phase as in Figure 21, and then move the mid point back data point by data point. The correlation coefficients will probably be relatively low for these first few tries. However, as one progressively moves backward toward the onset of the elimination phase, the correlation coefficient should increase slightly, with the slope increasing until a fairly uniform slope is achieved with fairly consistent correlation coefficients. As one proceeds back beyond the beginning of the elimination phase, the correlation coefficient will decrease and the elimination rates will increase.

For example, visual inspection of the ethanol concentration curve for Subject 612 shows that the graph changes slope at about 103 minutes. Starting least squares fitting for the data points between 119 minutes and 237 minutes, ß is -.197 mg/210 ml per minute, and r = -0.988 (Table 19.). Advancing to the data between 111 minutes and 237 minutes, r increases to -0.989 and ß increases to -0.199. Beginning at 103 minutes, r peaks at -0.991 and ß is -0.201. From this point onward, ß continues to increase dramatically with a concomitant decrease in Pearson's r.



MEAN Males = 73.4 +/- 25.5; Females = 70.2 +/- 25.7
SKEWNESS Males = .33; Females = .68

Figure 22. Time to diffusion equilibrium following consumption for both males and females. There was no statistical difference between the time required for males and females to reach diffusion equilibrium. (t= -.89, P = 0.372, r_{PB} = 0.03).

Time to Diffusion Equilibrium

The time to diffusion equilibrium from the end of drinking was very variable for both male (C of V = 34.7%) and female subjects (C of V = 36.6%). Figure 22. shows that for both the male and female subjects it took on average 73 minutes to reach diffusion equilibrium once consumption had ceased.

The time to diffusion equilibrium from the commencement of drinking appeared to be a function of the time required for both male and female subjects to consume their alcohol. Figure 23 shows the correlation ($r^2 = 0.2434$, $P < .001$) between the consumption period for the male subjects, and the time required to reach the beginning of the elimination phase. The correlation persisted even when the two data points on the far right of the graph were excluded.

Goodness of Fit for Linear Regression Analyses

Figure 24 shows the correlation coefficients for the lines fitted by linear regression to the elimination phase data points. A two tailed *t*-test for independent samples showed a statistical difference ($t = 2.49$, $P < .05$, $r_{PB} = 0.09$) between the mean correlation coefficient for males (-0.991) and for females (-0.994). However, the point biserial correlation coefficient indicates that less than 1% of the variance is due to gender. It can be seen from Figure 24 that there was excellent fit for the lines and that there was therefore a high degree of linearity between ethanol concentration and the time of breath sampling.

The Elimination Rate

Table 20 shows that the mean elimination rate ($ß_{60}$) for males was calculated to be 13.5 ± 2.5 mg/210 ml per hour. Using a two tailed *t*-test for independent sample means, this value was found to be statistically different ($P < .001$) from the mean rate for females, which was 15.2 ± 3.3 mg/210 ml per hour. However, comparison of these two means indicates that only 3% of the variance between male and female $ß_{60}$s can be explained by gender ($r_{PB} = 0.172$). The distribution of elimination rates was more positively skewed for the females than for males (Figure 25.). For both the male and the female subjects, $ß_{60}$ appeared to be statistically correlated to the age of the subject, albeit a very low correlation (Figure 26.), but did not

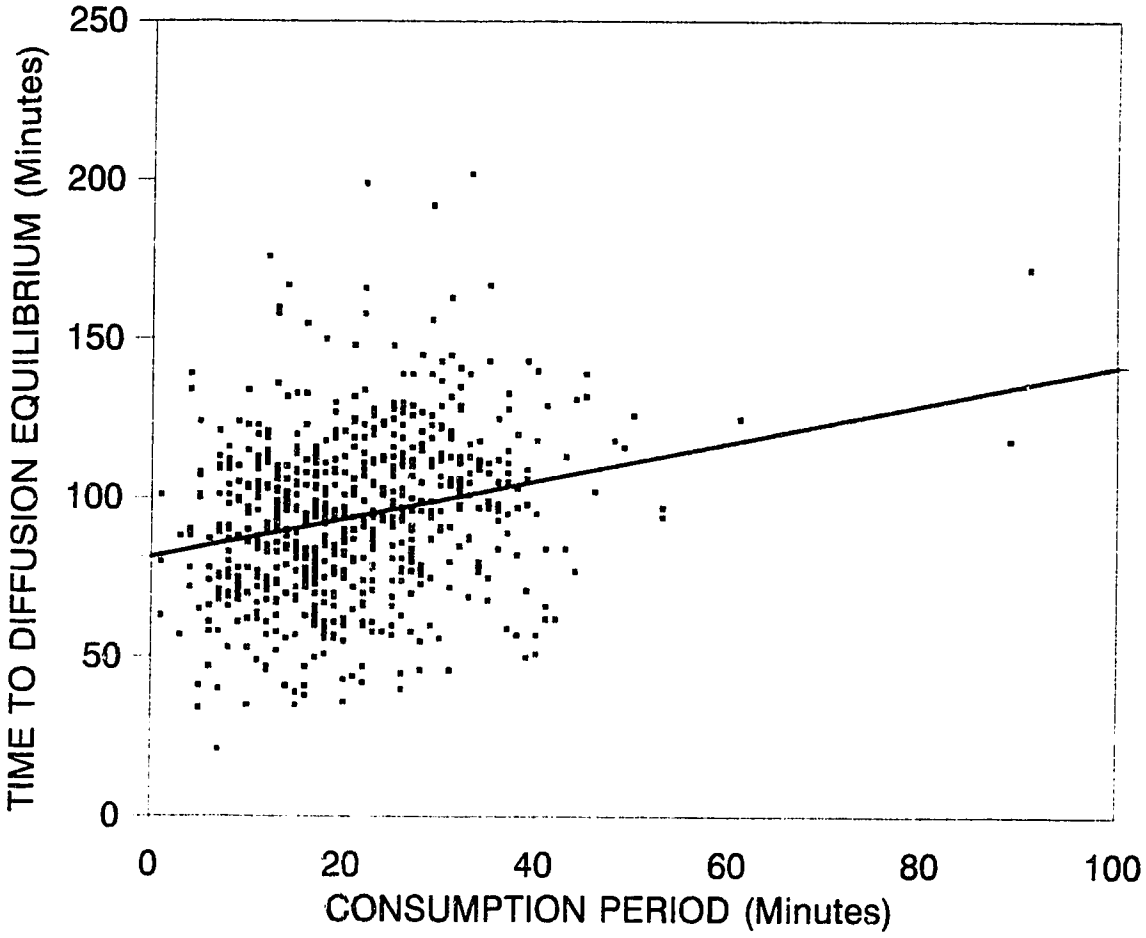


Figure 23. Correlation between the period of consumption, and the time from the beginning of consumption to diffusion equilibrium for the male subjects. r^2 = 0.2434, and P = 0.000 using a two tailed test of significance.

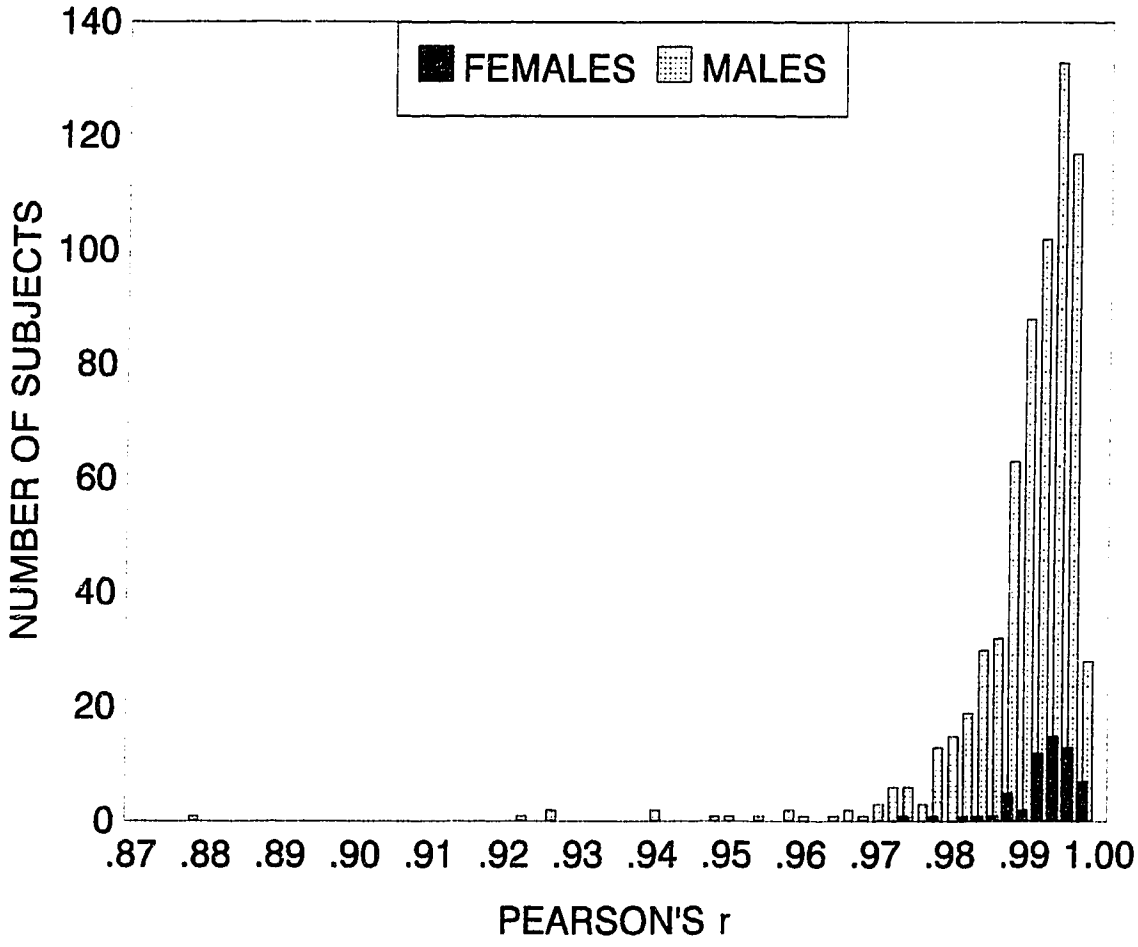


Figure 24. Correlation coefficient for the fitting of the linear regression line to the elimination phase concentration versus time data points. For females r = -0.994 ± 0.005 (range = -0.974 to -0.999) and for males r = -0.991 ± 0.009 (range = -0.878 to -0.999). Skewness for females and males was -2.04 and -5.04 respectively. Comparison of the mean $ß_{60}$s for males and females results in t = 2.49, P = 0.013, and r_{PB} = 0.09.

Table 20.

Elimination Rates for Males and Females

Parameters	Males	Females
N	676	59
β_{60} ± SD (mg/210 L breath/hour)	13.5 ± 2.51	15.2 ± 3.26
C of V (%)	18.6	21.4
Range	6.0 - 22.9	8.8 - 23.8
Skewness	0.565	0.754

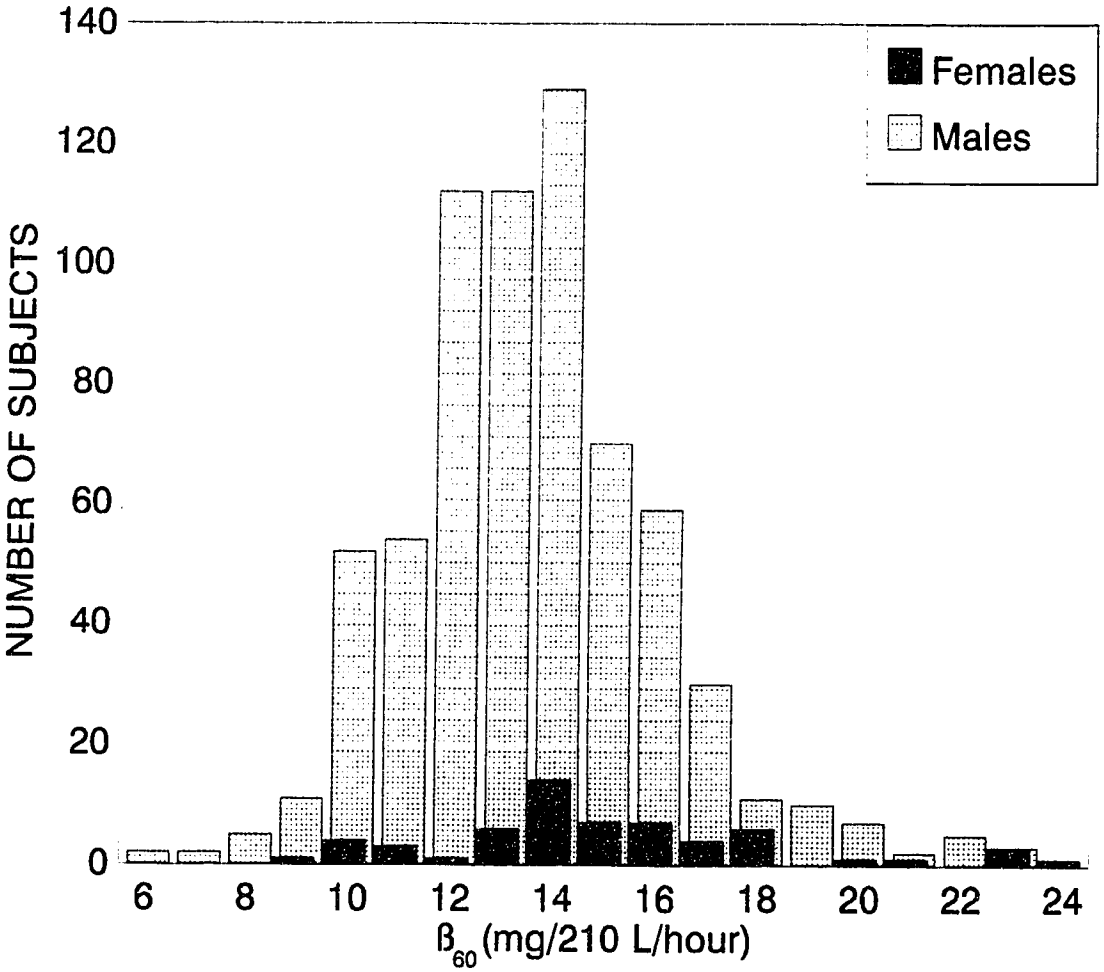


Figure 25. Distribution of elimination rates for males and females.

Table 21

Frequency of Elimination Rates for Males and Females

	Males		Females	
Elimination Rate (mg/210 L / hour)	**%**	**Cum %**	**%**	**Cum %**
6	0.3	0.3	0	0
7	0.3	0.6	0	0
8	0.6	1.2	0	0
9	1.7	2.9	1.8	1.8
10	6.5	9.4	7	8.8
11	8.9	18.3	5.2	14
12	15.4	33.7	0	14
13	17.7	51.4	10.6	24.6
14	16.9	68.3	22.8	47.4
15	12.6	80.9	14	61.4
16	7.9	88.8	12.3	73.7
17	5.5	94.3	7	80.7
18	1.7	96	10.5	91.5
19	1.5	97.5	0	91.5
20	0.8	98.3	0	91.5
21	0.5	98.8	1.8	93
22	0.7	99.5	0	93
23	0.5	100	5.2	98.2
24	0	100	1.8	100

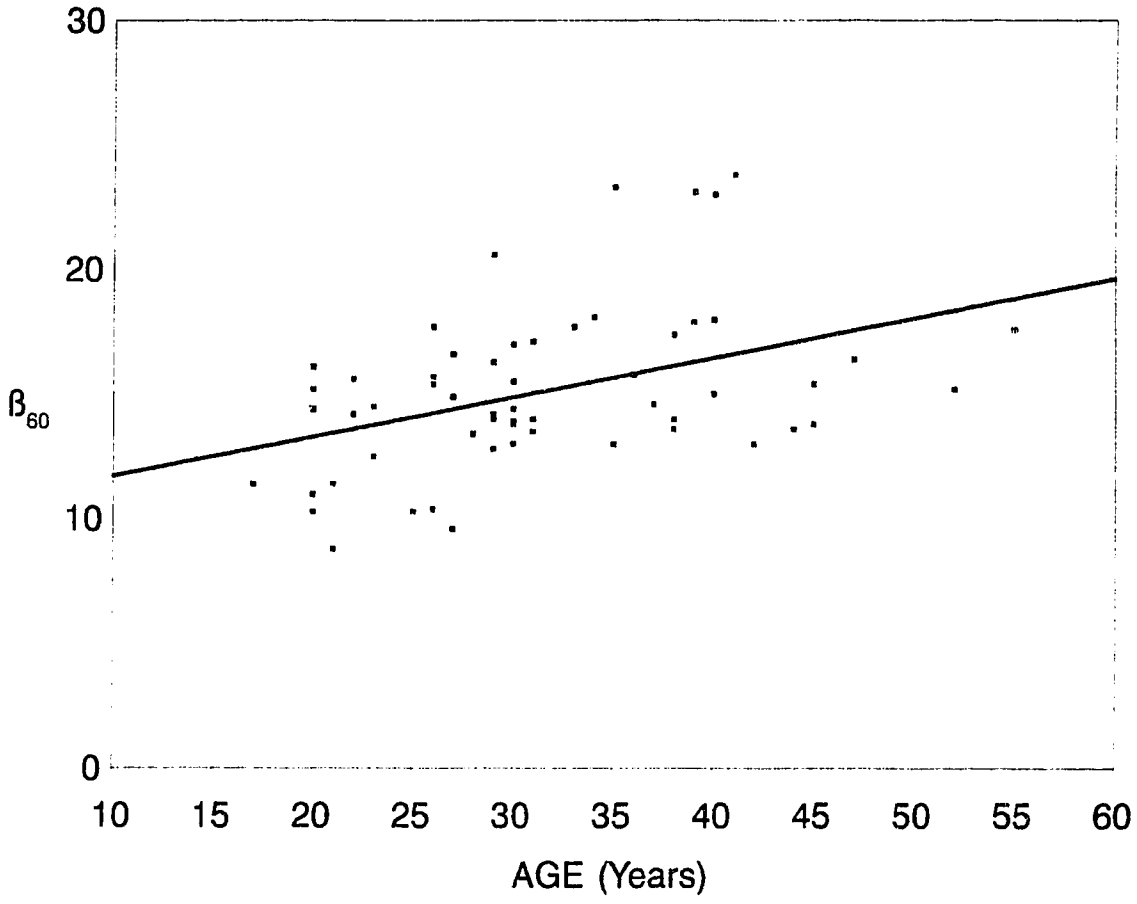


Figure 26. Correlation between the age of the female subjects and their $ß_{60}$(mg/210 L breath/hour). r^2 = 0.4199 (P <.05). For the male subjects r^2 = 0.1171 (P <.05) For females the average annual increase in their elimination rates is predicted to be 0.158 mg/210/hour; for males 0.025 mg/210 L/hour.

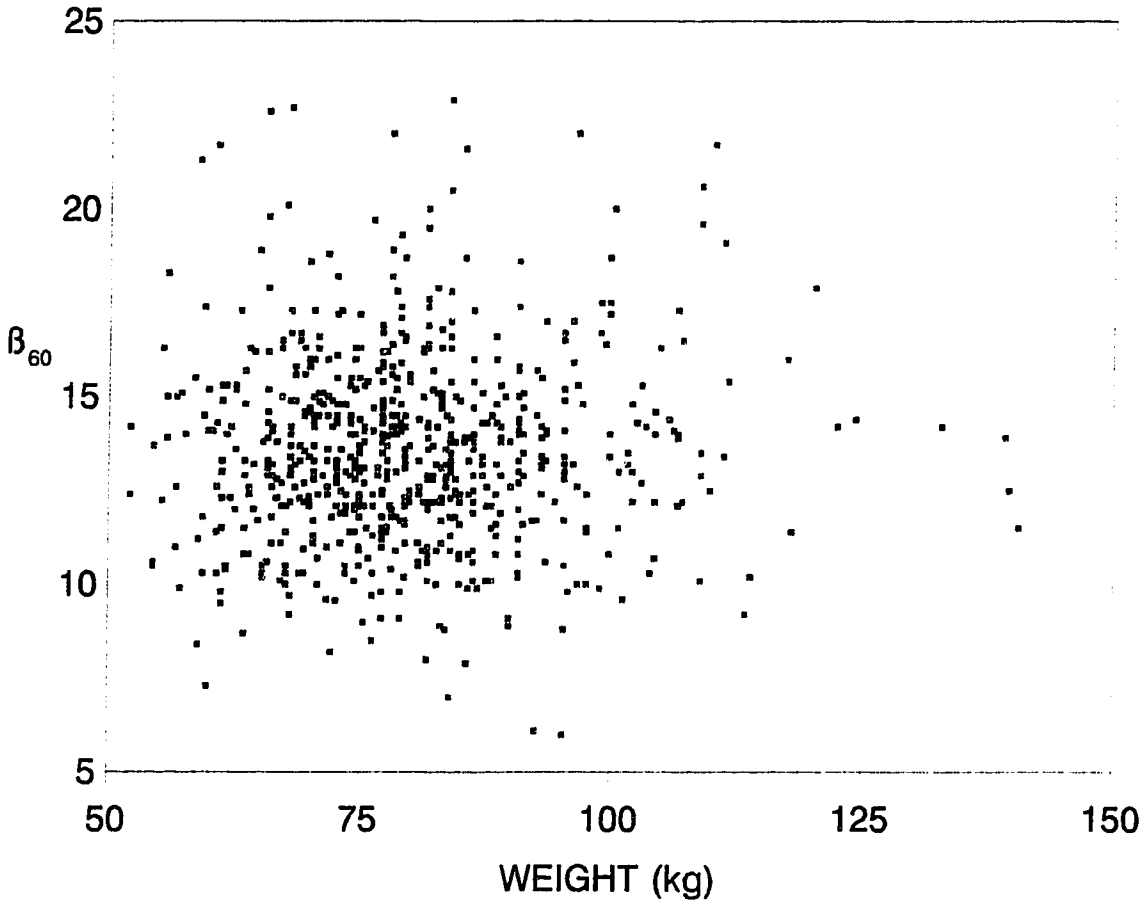


Figure 27. Correlation between the male subjects weights and $ß_{60}$ (mg/210 L breath/hour). r^2 = -0.044 (P >.05) and for females r^2 = 0.014 (P>.05).

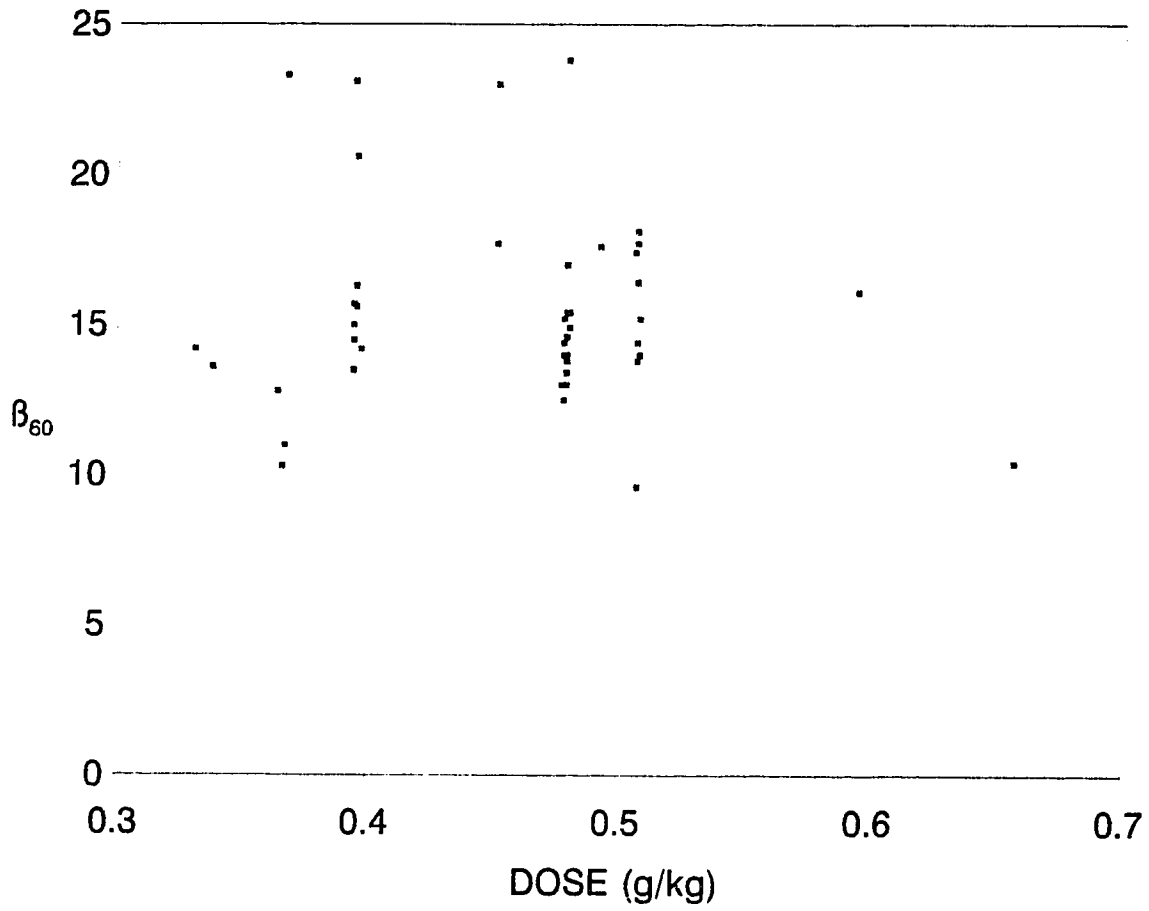


Figure 28. The correlation between dose and $ß_{60}$ (mg/210 L breath/hour) for the female subjects r^2 = -0.1622 (P >.05). For the male subjects r^2 = -0.1628 (P <.001).

not appear to be related to the weight of the subject (Figure 27.) nor their PI (Figure 28.). However, when the data for males and females was pooled, there was a negative correlation between weight and $ß_{60}$ (Figure 28.) but not between PI and $ß_{60}$.

Finally, Figure 28 shows that while there did not appear to be a significant correlation between $ß_{60}$ and the dose of ethanol consumed by the females ($P > .05$), this did not hold true for the male subjects where there was a statistically significant correlation between $ß_{60}$ and dose ($P < .001$). For the male subjects this is inconsistent with the findings of Mellanby[97], Widmark[98] and others, but it is in agreement with the conclusions of Eggleton[99] and Wagner[100]. It must be remembered however, that the comparison made by these statistics was between the range of doses given to all subjects in the sample and their collective $ß_{60}$ values. The statistic cannot be used to reject the hypothesis that for individual subjects, a change in dose has no effect on $ß_{60}$. Similarly, a correlation existed between $ß_{60}$ and C_0 for the males subjects ($r^2 = .1730$, $P < .001$) and the female subjects ($r^2 = .3119$, $P < .05$).

Volume of Distribution (Widmark's r Factor)

The volume of distribution is calculated by dividing the dose of alcohol consumed (gm anhydrous alcohol / kg) by C_0 (gm/L). The value for Widmark's *r* factor was 0.75 ± 0.09 L/kg for males and 0.65 ± 0.11 L/kg for the female subjects (Figure 29.). This difference was found to be statistically significant ($P < .001$). However, only 7% of the variance between the mean of Widmark's *r* for males and for females can be explained by gender, the other 83% in difference is unaccounted for. These values of *r* compare favorably, albeit slightly higher, with Widmark's original values of 0.68 ± 0.085 for males, and 0.55 ± 0.055 for his female subject[101].

Summary of Group I Subject Data

Table 22 summarizes the relationships which exist between the characteristics of the Group I subjects and the various pharmacokinetic parameters.

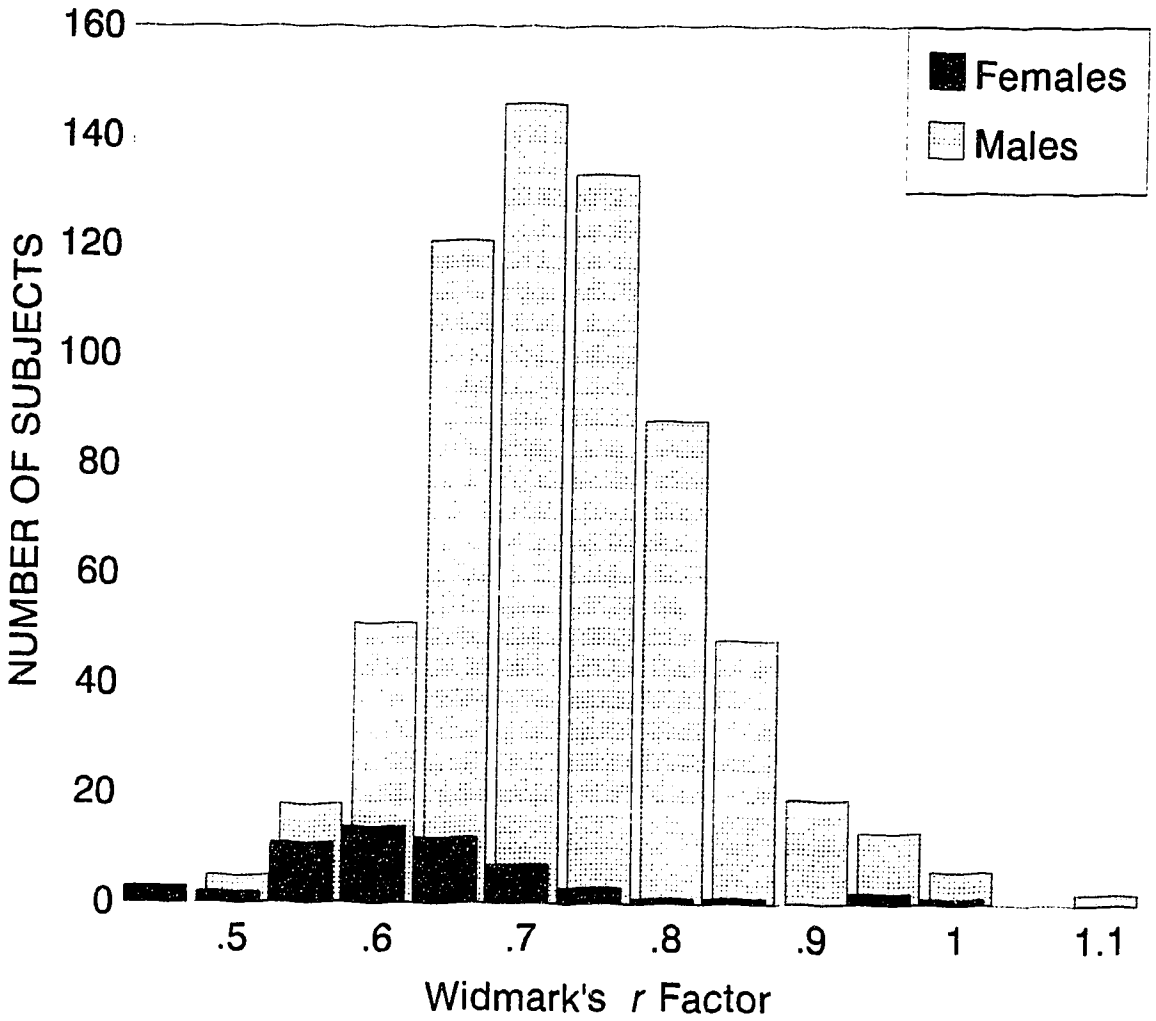


Figure 29. Widmark's *r* factor for females = 0.652 ± 0.113 L/kg (Range = 0.45 to 1.05), skewness = 1.41. For males the *r* factor = .747 ± 0.093 L/kg (Range = 0.50 to 1.14), skewness = 0.540. Comparison of the two sample means provides $t = 7.27$, $P < .001$, $r_{PB} = 0.264$.

Table 22

Correlations Between Group I Individual Characteristics And Pharmacokinetic Parameters

		0.19	-0.029	0.047	0.071	0.051	0.234	-0.222	0.127
	0.19		0.486	0.079	0.156	0.06	0.241	-0.19	-0.08
	-0.029	0.486		0.15	0.057	-0.009	0.008	0.149	-0.182
	-0.209	-0.948	-0.283	-0.079	-0.163	-0.077	-0.267	0.224	0.068
	0.047	0.079	0.15		0.356	0.198	0.595	0.354	-0.199
	0.071	0.156	0.057	0.336		0.243	0.285	0.045	-0.069
	0.051	0.06	-0.009	0.198	0.243		0.165	0.023	-0.22
	0.031	-0.003	-0.037	0.06	-0.165	0.915	0.055	0.065	-0.193
	0.234	0.241	0.008	0.595	0.285	0.165		0.528	0.181
	-0.222	-0.19	0.149	0.354	0.045	0.023	-0.528		-0.413
	0.127	-0.08	-0.182	-0.199	-0.069	-0.22	0.181	-0.413	

Note: *Consume to DE* refers to the interval between the commencement of consumption and DE. *Time to DE* refers to the interval between the end of consumption and DE. Based upon the 2-tailed Student *t* test for significance, correlations existing between paired items ($P < .05$) are shown in the yellow-shaded areas.

Results and Discussion for Group II Subjects

Characteristics of Sample and Comparison to Group I

Table 23 provides information about each of the 29 subjects who were tested on two separate days to determine if there was a significant intrasubject change in the rate of elimination, as well as other associated parameters. Of the 29 subjects, two were female; 27 were male. These subjects ranged in age from 18 to 59. While such characteristics such as age and weight varied, it is not surprising that height remained robust.

There was no statistical significance ($P > .05$ for all comparisons) between the age, weight and height of the Group I subjects and their Group II counterparts (Table 24).

Dose and Period of Consumption of Ethanol

The dose of 95% by volume alcohol administered to the subjects, mixed with orange juice, is provided in Table 25. On average, consumption on the first day of testing lasted 22 ± 7 minutes for the male subjects and 27 ± 4 minutes for the female subjects. There was no significant difference between these two averages ($P > .05$). During the second day of testing, the female subjects again took longer on average to drink their cocktails (26 ± .7 minutes) than the male subjects who took on average 20 ± 10 minutes, but again there was no significant difference in the time required for the male and female subjects to consume their alcohol.

Breath Alcohol Concentration Curves and Goodness of Fit

Appendix C shows the resulting breath alcohol concentration profiles for each of the 29 subjects. Each subject's graph has the alcohol concentration curve from the second day of drinking overlaid on the first day's curve for comparison purposes. The mean correlation coefficient for day 1 graphs was -0.9895 and for day 2 it was -0.9900. Comparison of the correlation coefficients for day one and day two showed that there was no statistical difference between the two means ($t = -.37$, $P > .05$). As well, there was no statistical difference between the correlation coefficient values for Group I and II subjects ($t = .77$, $P > .05$).

Table 23

Physical Characteristics

Of Group II Subjects

Subject	Gender	Interval (Days)	Age		Weight (kg)		Height (m)	
			Day 1	Day 2	Day 1	Day 2	Day 1	Day 2
1	F	7	24	24	54.4	56.7	1.54	1.54
2	F	3	34	34	56.7	56.2	1.73	1.73
3	M	21	28	28	77.6	77.1	1.75	1.75
4	M	469	23	24	70.8	67.1	1.75	1.75
5	M	26	48	48	87.1	88	1.82	1.82
6	M	11	32	32	88	88	1.78	1.78
7	M	14	35	35	74.4	73	1.83	1.83
8	M	10	35	35	66.2	66.2	1.78	1.78
9	M	7	36	36	68	70.3	1.73	1.73
10	M	63	44	44	73.9	74.4	1.85	1.85
11	M	37	23	24	61.2	60.3	1.68	1.68
12	M	2,042	32	35	78.5	84.8	1.73	1.73
13	M	11	18	18	72.6	71.7	1.78	1.78
14	M	74	41	41	97.5	97.1	1.93	1.93
15	M	42	44	44	75.3	78.5	1.8	1.8
16	M	56	34	34	69.4	68	1.83	1.83
17	M	41	26	26	72.1	70.3	1.68	1.68
18	M	27	59	59	117.5	116.1	1.85	1.85
19	M	13	33	33	87.1	87.5	1.78	1.78
20	M	13	30	30	75.3	72.6	1.75	1.75
21	M	14	30	30	69.8	70.3	1.8	1.8
22	M	132	43	43	66.7	63.5	1.79	1.79
23	M	12	39	39	76.7	74.4	1.7	1.7
24	M	31	35	35	83.9	83.5	1.78	1.78
25	M	13	36	36	82.1	82.6	1.75	1.75
26	M	13	30	30	73.9	74.8	1.78	1.78
27	M	1,261	23	27	95.2	95.3	1.88	1.88
28	M	3	18	18	68	66.2	1.73	1.73
29	M	157	41	41	67.6	66.2	1.7	1.7
Average ± SD		160 ± 443	34 ± 9	34 ± 9	76.1 ± 12.9	75.9 ± 13.1	1.77 ± 0.07	1.77 ± 0.07

Table 24.

Comparison of Group I and Group II

Physical Characteristics.

Subject Group	Age (years)	Weight (kg)	Height (m)
Group I	34.0 ± 11.1[a]	78.8 ± 13.6[c]	1.77 ± .08[e]
Group II - Day 1	33.6 ± 9.2[a, b]	76.1 ± 12.9[c, d]	1.77 ±.07[e, f]
Group II - Day 2	33.9 ± 9.0[b]	75.9 ±13.1[d]	1.77 ± .07[f]

Note: *t* - test values between alphabetic superscripts are, [a,a] $t = .30$, $P = .762$, $r_{PB} = .011$; [b,b] $t = -.180$, $P = .083$, $r_{PB} = .327$; [c,c] $t = .92$, $P = .359$, $r_{PB} = .034$; [d,d] $t = -.50$, $P = .619$, $r_{PB} = .095$; [e,e] $t = -.28$, $P = .779$, $r_{PB} = .010$; [f,f] $t = -1.32$, $P = .198$, $r_{PB} = 1.00$

Table 25.

Pharmacokinetic Parameters

For Group II Subjects

	Dose (ml/kg 95%)		Consumption (Min.)		Time To DE (Min.)		C_0 (mg/210 L Breath)		β_{60} (mg/210 L per hour)		Widmark's r Factor (L/kg)	
Subject	Day 1	Day 2	Day 1	Day 2	Day 1	Day 2	Day 1	Day 2	Day 1	Day 2	Day 1	Day 2
1	0.672	0.672	30	26	56	101	80	78	14	11	0.63	0.65
2	0.672	0.672	24	25	93	99	76	77	20	17	0.66	0.65
3	0.738	1.117	19	55	104	141	64	91	16	15	0.87	0.92
4	0.83	0.831	33	27	139	102	69	83	10	11	0.91	0.75
5	0.784	0.785	30	24	122	91	87	80	11	13	0.68	0.74
6	0.785	0.785	26	25	139	113	73	71	13	13	0.8	0.82
7	0.784	0.785	35	22	167	100	79	78	12	11	0.74	0.76
8	0.785	0.785	18	17	61	101	100	92	16	16	0.59	0.64
9	0.784	0.785	18	18	67	82	62	83	14	16	0.94	0.71
10	0.784	0.785	26	16	111	78	83	88	12	13	0.71	0.67
11	0.784	0.785	18	19	108	50	70	75	14	17	0.84	0.79
12	0.83	0.784	15	22	84	84	82	71	14	11	0.76	0.82
13	0.784	0.785	14	28	100	106	78	74	11	10	0.75	0.8
14	0.784	0.784	23	27	86	112	85	80	15	13	0.69	0.74
15	0.784	0.784	10	11	90	106	80	79	14	13	0.74	0.75
16	0.785	0.785	19	8	76	38	84	68	20	17	0.7	0.86
17	0.785	0.785	33	15	105	115	77	82	14	12	0.77	0.72
18	0.674	0.682	38	39	103	75	78	86	15	13	0.64	0.59
19	0.784	0.785	21	13	119	89	68	72	9	11	0.87	0.82
20	0.784	0.784	26	14	78	90	84	82	12	11	0.7	0.72
21	0.785	0.785	14	14	96	71	78	68	12	12	0.75	0.67
22	0.784	0.787	12	19	89	109	74	69	13	13	0.76	0.86
23	0.784	0.785	14	11	98	92	71	74	13	13	0.8	0.85
24	0.784	0.785	17	17	103	115	74	84	10	12	0.83	0.79
25	0.784	0.785	22	20	91	100	82	80	12	13	0.8	0.7
26	0.744	0.785	22	19	99	91	79	80	11	12	0.72	0.73
27	0.829	0.784	25	10	125	68	71	72	10	13	0.74	0.73
28	0.785	0.785	23	20	111	33	74	80	13	14	0.88	0.81
29	1.154	0.785	28	20	104	70	115	88	12	13	0.79	0.74
Average	0.79	0.79	23	21	101	90	79	79	13	13	0.76	0.8
± SD	0.7	0.8	7	9	24	24	10	7	3	2	0.09	0.08

Time to Diffusion Equilibrium

The mean time for Group II subjects to achieve DE from the commencement of drinking on day one was 101 ± 24 minutes (Table 25.). This was not significantly different from the Group I subjects whose average time was 94 ± 26 minutes (t = -1.34, P > .05, r_{PB} = .00245). Similarly, there was no significant difference between the time for the Group II subjects to achieve DE on day one as compared to day two (mean = 91± 24, t = -1.53, P > .05, r_{PB} = .282).

The Elimination Rate

Table 25 shows that the mean elimination rate for day one and day two was 13 mg/210 L /hour. There was no significant difference between these two values (t = .26, P > .05, r_{PB} = .050). Nor was there a statistical difference between the Group II day one average and the average elimination rate for the Group I subjects (mean = 13.7, t = 1.03, P > .05, r_{PB} = .194). The distribution of the intrasubject variability of $ß_{60}$ is shown in Figure 30.

Table 26 shows that the difference between the rates of elimination for day one and day two was not a function of age (P = 0.1143), the amount consumed (P = -0.319), the interval between the first day and the second day of testing (P = 0.0596), or C_0 (P = 0.1601).

Figure 31 shows the correlation between $ß_{60}$ values for day one and day two. Such correlations depend upon one of the sets of values, *i.e.* the day one values, being the dependent variable with the other set the independent variable. To eliminate bias, two correlations were calculated; one assigning day one as the dependent variable (blue dashed line), the second with day two as the dependent variable (green dotted line). The average of these two correlation is represented by the solid black line which has a slope of 0.744, a y intercept of 3.36 and a correlation coefficient of 0.7122 (P < .001).

There was, however, a negative correlation between the daily rate difference and the time to achieve DE (r = -0.4074, P < .05) and the subjects' volume of distribution (r = -.5155, P < .05). A positive correlation (Figure 32.) was shown to exist between the day to day difference (Day 1 $ß_{60}$ - Day 2 $ß_{60}$) and the day one rate of elimination (r = 0.6704, P < .001); there was a negative correlation between the day to day difference and the second day rate (r = -0.0434, P = 0.823).

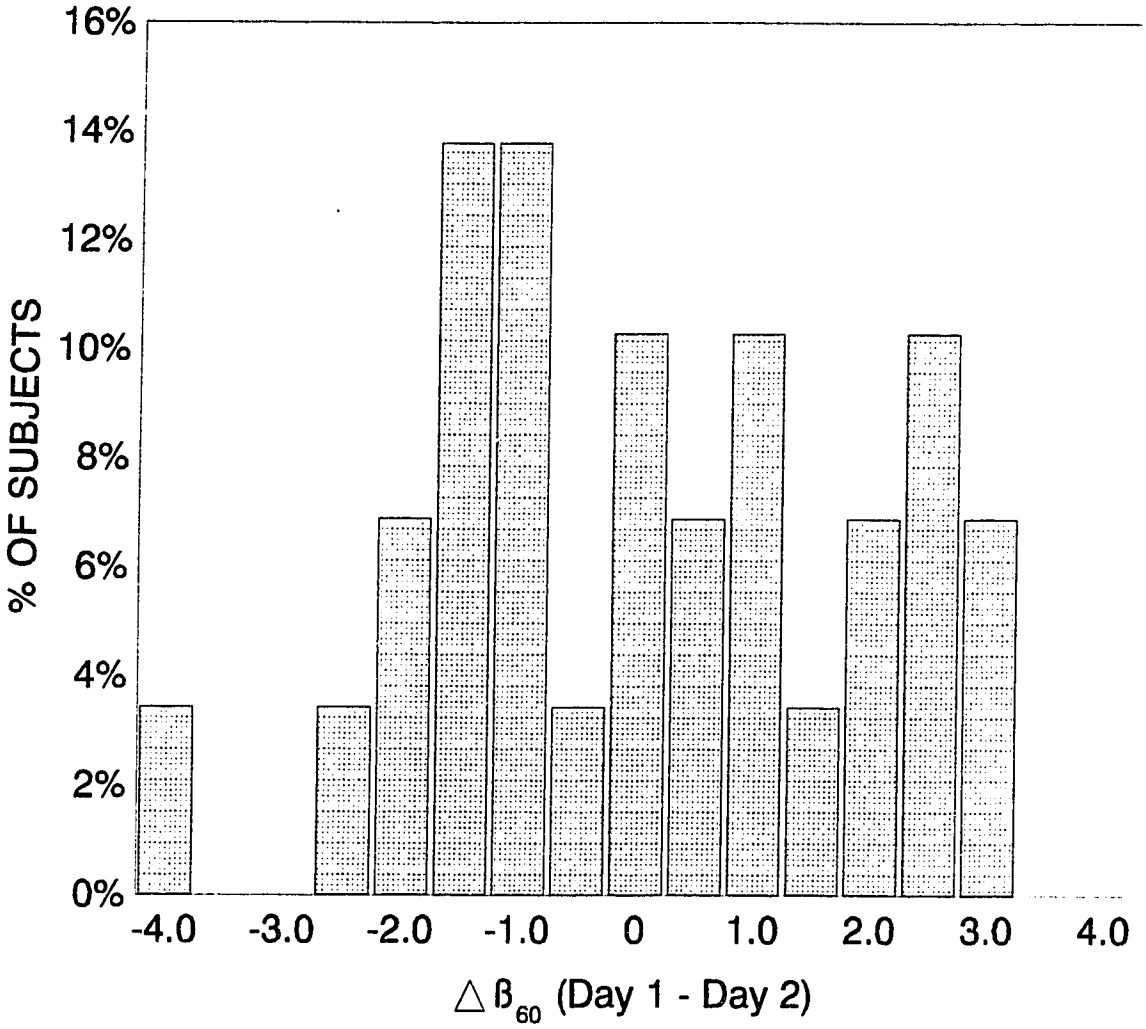


Figure 30. The distribution of the day to day difference in $ß_{60}$ values for all 29 subjects. The mean difference was 0.05 ± 1.82, with a range of -3.8 to 3.2 mg/210 L breath/hour.

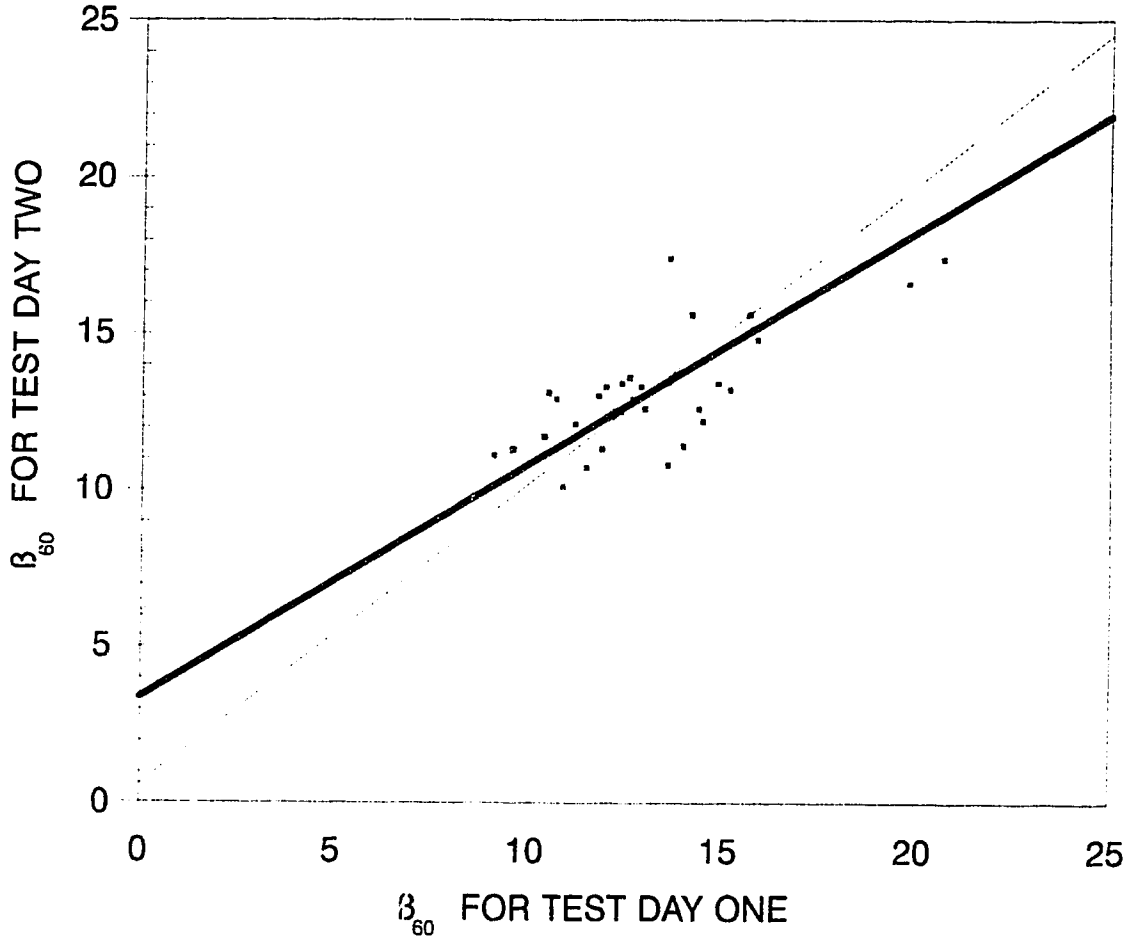


Figure 31. Correlation between paired $ß_{60}$ values for day one and day two of testing. The green dotted line is the correlation between the day two rate as the dependent variable and the day one rate as the independent variable. The formula for the line is $y = 0.5289x + 6.144$. The blue dashed line is the correlation between day one as the dependent variable and day two as the independent variable for which the formula is $y = 0.9589x + 0.591$. The average of these two correlations is represented by the black solid line whose formula is $y = 0.7439x + 3.367$. This shows a positive correlation ($P < .001$) between the subject's elimination rate on day one and on day two. The correlation coefficient was 0.7122 .

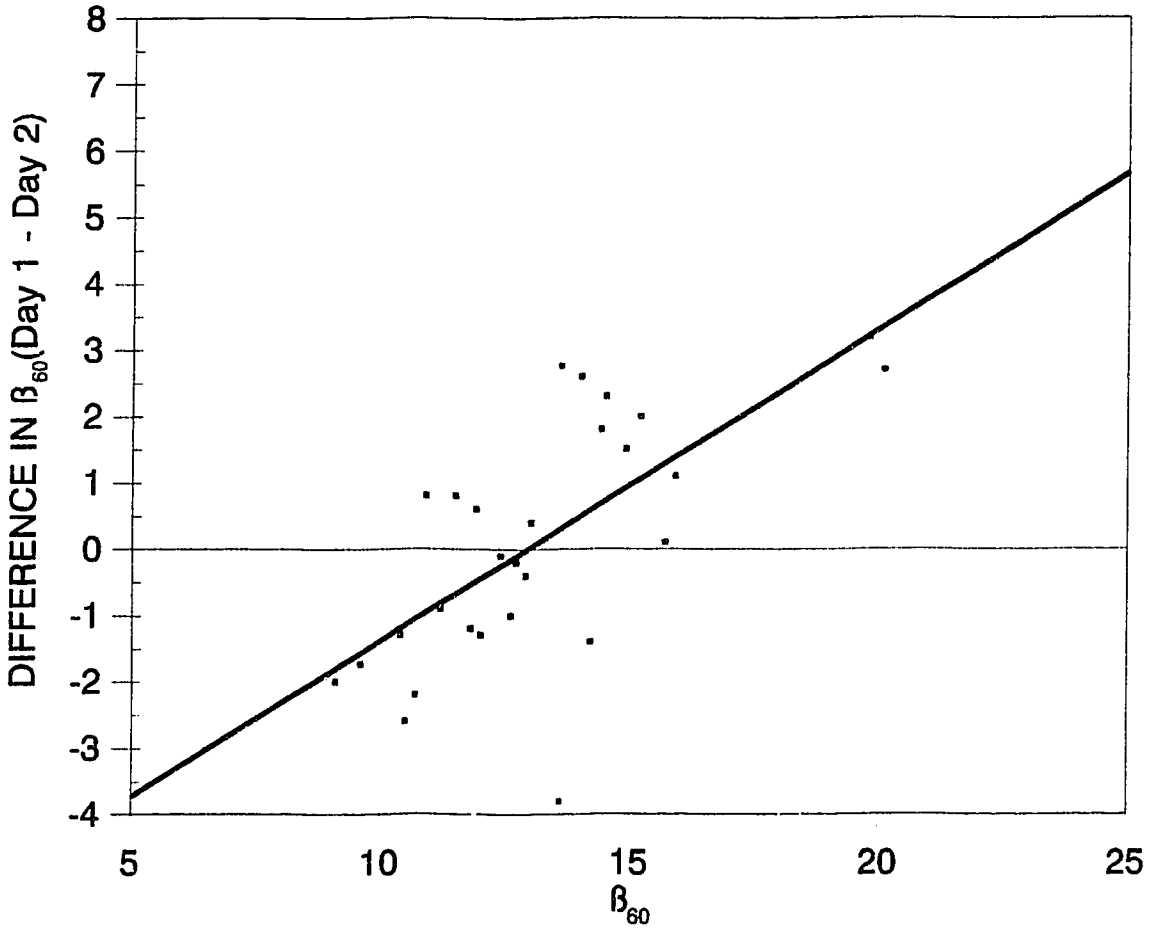


Figure 32. Correlation between the difference in the elimination rates from day one to day two and the day one elimination rate. This shows a positive correlation of 0.6704 ($P<.001$)

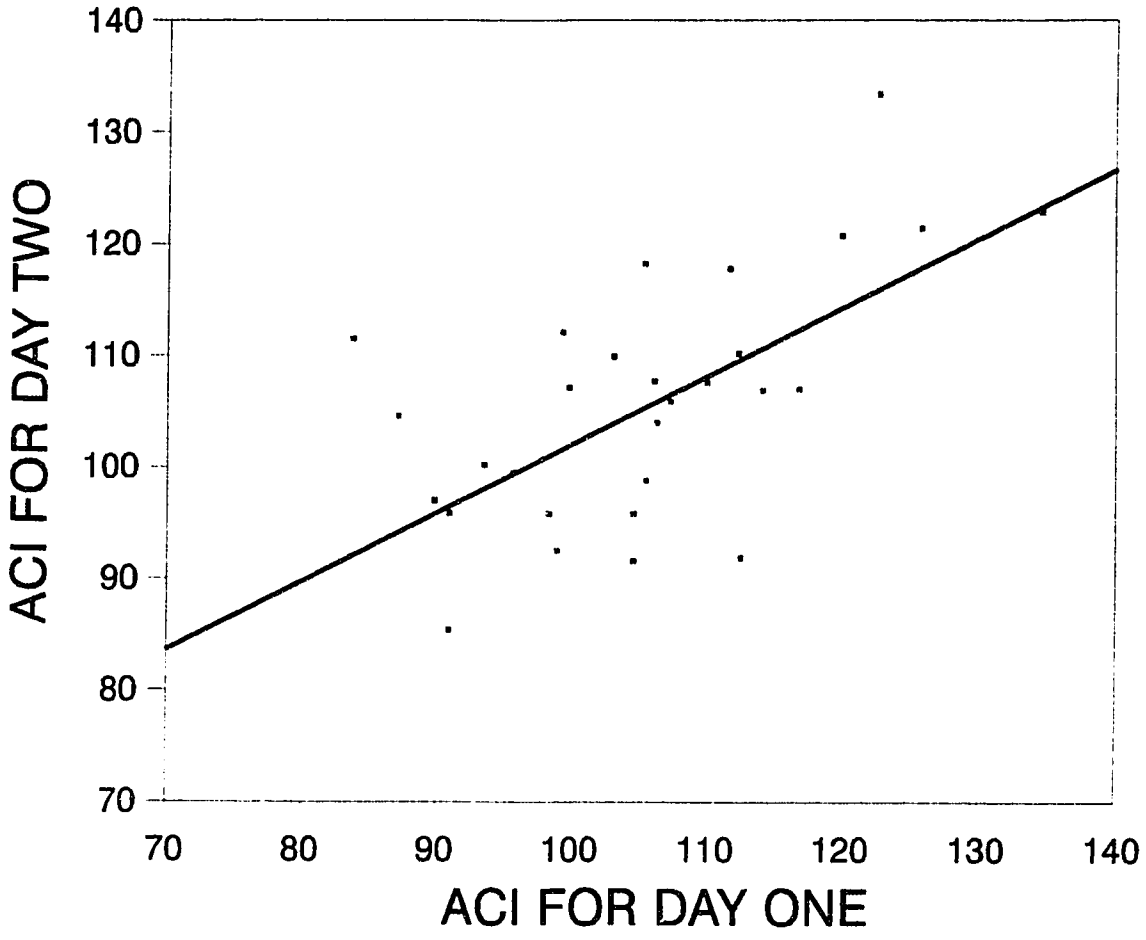


Figure 33. Testing the correlation between the paired values of ACI for the two days of testing showing a positive correlation ($r^2 = 0.6119$, $P<.001$)

Alcohol Concentration Index

The alcohol concentration index (ACI) is defined as the expected blood or breath alcohol concentration that would be generated for each ml of 100% (v/v) ethanol per kg of body weight. It is calculated by dividing the subject's C_0 value by the dose of alcohol consumed. Figure 33 shows the correlation between the individual ACI values for day one and day two. Similar to the statistical treatment described for Figure 31, Figure 33 shows the average of the two correlations determined. The blue line is defined by the expression $y = 0.6137x + 40.8$. Clearly, there is a positive correlation between the ACI values for day one and day two ($P<.001$) with a correlation coefficient of 0.6119.

Volume of Distribution - Widmark's r Factor

Table 25 shows the Widmark r factor values for the Group II subjects on day one and day two. There was no significant difference between the mean r factor values for the subjects from the first day to the second day of testing ($t = -0.52$, $P > .05$, $r_{PB} = 0.0413$). Not surprisingly however, the female subjects had a significantly lower average r factor than their male counterparts ($t = -2.10$, $P < .05$, $r_{PB} = 0.100$). Figure 34 shows that a positive correlation ($r = 0.5404$, $P < .05$) existed between the Widmark r factor values for day one and day two. Similar to Figures 31 and 33, the blue line in Figure 34 represents the average of the two correlations determined. The formula for the blue line is $y = 0.5430x + 0.3458$.

Accuracy in Estimating Day Two Alcohol Concentrations Based Upon Day One Data

For each of the day two alcohol concentration profiles (Appendix C), a sample time was computer generated at random between 100 and 200 minutes. The actual alcohol concentration corresponding to that sample time was then calculated as the net difference between the day two C_0 value and the product of the time and the day two ß value (the rate of elimination per minute) for that profile.

The predicted alcohol concentration was calculated by multiplying the day one ACI value by the dose of alcohol consumed on day two. The result provided the estimated gross blood alcohol concentration for day two. From this value was subtracted the

amount of alcohol predicted to be eliminated based upon the assigned time and the day one elimination rate. The difference between the gross alcohol concentration and the amount expected to be eliminated provided the predicted net alcohol concentration for day two.

The actual alcohol concentration calculated for day two was then subtracted from the predicted value and the difference expressed as the percentage of the actual alcohol cncentration. Table 27 shows the frequency of the differences. The mean difference for the 29 random comparisons made was -0.020 ± 17.5%. The Table further shows that for 80% of the time, the predicted alcohol concentration was no greater than 10% higher than the actual day two concentration.

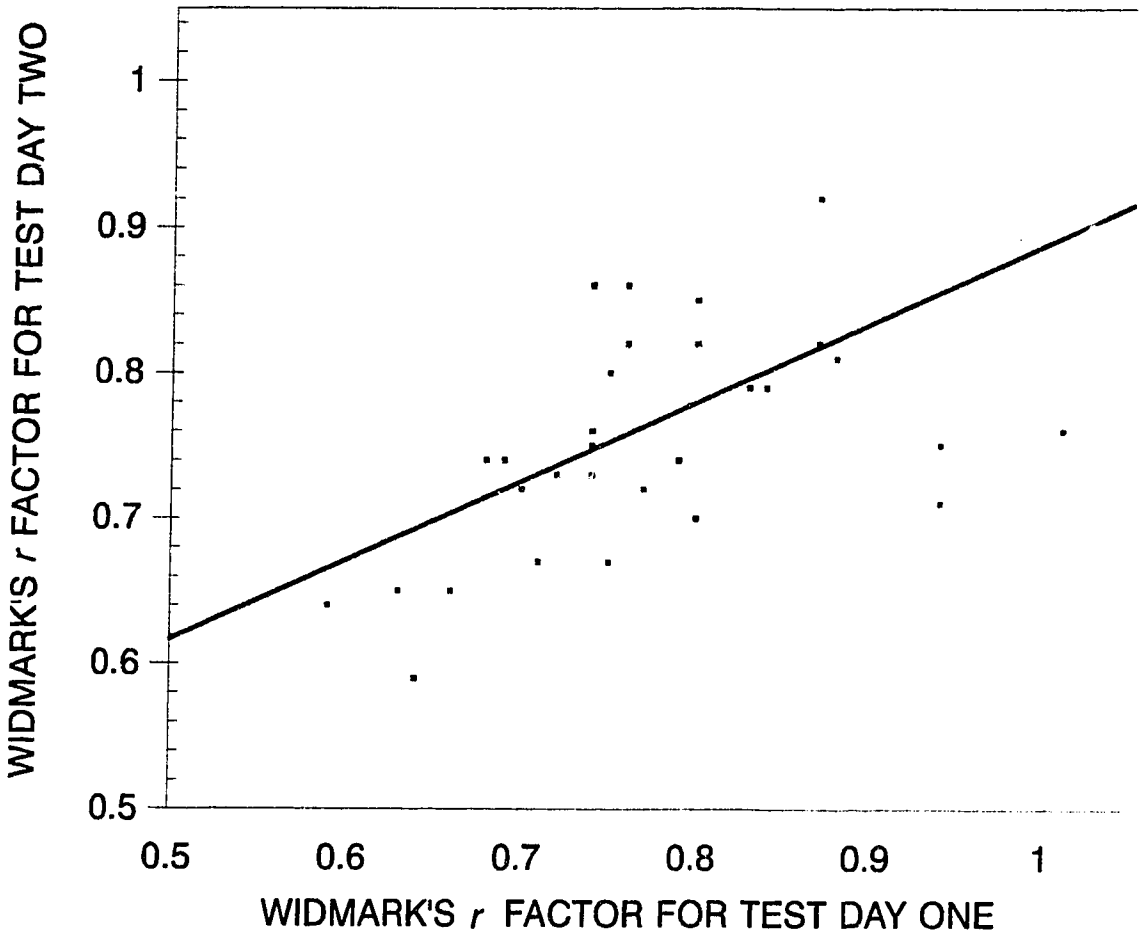


Figure 34. Correlation between paired Widmark *r* factor values for day one and day two. The correlation coefficient was 0.5404 ($P < .05$).

Table 26

Correlations Between Group II Subjects' Characteristics And Their Pharmacokinetic Parameters

	0.4813	0.4152	0.0092	0.1227	-0.0619	0.3093	-0.3635	0.16	0.1143
0.4813		0.6475	0.0847	0.2786	0.3041	-0.0834	0.0066	-0.2332	0.0865
0.4152	0.6475		-0.0061	-0.0035	0.3171	0.0376	-0.0532	-0.0598	-0.1072
-0.3749	-0.957	-0.4875	-0.0016	-0.2444	-0.3152	0.115	-0.1231	0.3639	0.1349
0.0092	0.0847	-0.0061		-0.0166	0.1622	0.5845	0.2175	-0.2905	-0.319
0.1227	0.2786	-0.0035	-0.0166		0.4328	0.1506	-0.208	-0.0977	0.0007
-0.0619	0.3041	0.3171	0.1622	0.4328		-0.2192	0.3709	-0.4696	-0.4074
-0.109	0.2409	0.3494	0.1837	0.141	0.9535	-0.2911	0.4768	-0.4831	-0.4476
0.3093	-0.0834	0.0376	0.5845	0.1506	-0.2192		-0.6573	0.1127	0.1601
-0.3635	0.0066	-0.0532	0.2175	-0.208	0.3709	-0.6573		-0.3872	-0.5155
0.16	-0.2332	-0.0598	-0.2905	-0.0977	-0.4696	0.1127	-0.3872		0.6704
0.1143	0.0865	-0.1072	-0.319	0.0007	-0.4074	0.1601	-0.5155	0.6704	
-0.1563	0.1631	0.0647	0.2082	-0.0882	0.0412	-0.0091	-0.5155	-0.125	0.0596

Note: *Consume to DE* refers to the interval between the commencement of consumption and DE. *Time to DE* refers to the interval between the end of consumption and DE. Based upon the 2-tailed Student *t* test for significance, correlations existing between paired items ($P < .05$) are shown in the yellow-shaded areas.

Table 27

Error in Predicting Day Two Alcohol Concentrations Based Upon Day One Data

Error	Frequency	%	Cum %
-42	1	3.4	3.4
-25	1	3.4	6.9
-23	1	3.4	10.3
-18	1	3.4	13.8
-17	1	3.4	17.2
-14	1	3.4	20.7
-12	1	3.4	24.1
-11	1	3.4	27.6
-10	1	3.4	31
-7	1	3.4	34.5
-6	1	3.4	37.9
-3	1	3.4	41.4
-1	1	3.4	44.8
0	1	3.4	48.3
1	1	3.4	51.7
2	2	6.9	58.6
5	1	3.4	62.1
6	3	10.3	72.4
7	1	3.4	75.9
8	2	6.9	82.8
18	1	3.4	86.2
20	1	3.4	89.7
21	1	3.4	93.1
31	1	3.4	96.6
45	1	3.4	100

When your horse dies,

Get off.

- Old Cowboy Wisdom

3. Discussion and Conclusions

The major purpose of this thesis was to measure the rates of alcohol elimination in drivers who have been charged with impaired driving, and to determine whether these rates vary from one individual to the next, and within the same individual. As a consequence, it was necessary to develop a protocol which would measure the elimination rates in an objective and reproducible way, something which to date has not been accomplished. This thesis describes such a protocol and reports the testing results of the largest sample size of persons who have been charged by the police with impaired driving.

From a forensic perspective, it is clear that the best means of mathematically describing alcohol elimination in humans will remain controversial for some time to come. It was never the intention of this thesis to resolve this issue. However, with the large sample size it is possible to define the parameters of the changes in human blood alcohol concentrations more clearly, and apply them to either linear Widmark elimination kinetics, or non-linear Michaelis-Menten metabolism kinetics.

Rates of Elimination

Diffusion Equilibrium

Since the time of Widmark (1932), researchers have been warned that distribution equilibrium (DE) must have been achieved before reliable measurements of elimination rates can be made. Researchers such as Haggard and Greenberg[102]; Newman *et al.*[103]; Bruno *et al.*[104] were careful to ensure that a post absorptive equilibrium had been achieved between alcohol in the blood and the surrounding tissues before commencing with measurements for the rate of elimination. Others have waited some arbitrary period of time following consumption for what they presume to allow for DE to occur. Lundquist and Wolthers[105], and Passanti *et al.*[106] waited an arbitrary 120 minutes. Vesell *et al.*[107] and Wilson and his co-workers[108] waited 90 minutes, while Cole-Harding *et al.*[109] waited only 60 minutes following the last dose to assume that DE had been achieved. For others such as Eggleton[110], Shumate *et al.*[111], and Wagner and Patel[112], there is no clear indication where

they assumed, if in fact they made any assumptions at all, when DE occurred. Nagoshi and Wilson were more variable in their assumptions concerning the beginning of the elimination phase. For the initial testing they chose to wait 60 minutes from the last topping dose of alcohol; for the retest of their subjects, they chose to wait 20 minutes following the peak alcohol concentration before assuming the subject was in the elimination phase[113].

Based upon the results of this thesis, the average time to achieve DE following consumption of the alcohol was 73.3 ± 25.4 minutes (see Figure 22). Under the conditions of Nagoshi and Wilson's protocol, only 29% of the current subjects would have achieved DE. For the other 71%, the rate of decline that Nagoshi and Wilson identified as $ß_{60}$ would have been a hybrid of the rate of diffusion of alcohol from the blood into the surrounding tissues combined with the actual hourly rate of elimination of ethanol from the blood. Understandably, values of $ß_{60}$ achieved would be erroneously high. In fact Nagoshi and Wilson's mean value for $ß_{60}$ was 19.5 mg% per hour. For the Group I subjects tested for this thesis, Table 20 shows a mean $ß_{60}$ for males of 13.5 and for females of 15.2. Nagoshi and Wilson's $ß_{60}$ values are statistically higher (t = -12.20, $P < .001$, $r_{pB} = 0.41$) than those for the Group I subjects.

This hybrid of post absorption distribution/elimination combined with the elimination phase may explain in part the dramatic increase in the rate of decline in the experiments conducted by Eggleton[114]. During those experiments, in which Eggleton questioned whether the metabolic rate of alcohol was a function of alcohol concentration, an initial period of constant infusion of alcohol into his two cats resulted in a continuous increase in the plasma alcohol concentration (Figure 6). However, when a dose of alcohol was injected that significantly increased the plasma ethanol concentration from between 10 and 20 mg% to well in excess of 310 mg%, the plasma ethanol concentration went into a continuous decline even though the initial infusion rate was kept constant. The difference observed by Eggleton between the increasing alcohol concentration in the first half of the experiment and the rapid decline during the second half, cannot be assumed to be due to a change in the metabolic rate only. There are other, non-metabolic, variables involved.

During the initial infusion period, the plasma alcohol concentration in the one cat did not exceed 10 mg%, and in the other cat, did not exceed 25 mg%. Under these conditions of insignificant alcohol

concentrations, distribution of alcohol from the plasma into the tissues would be almost instantaneous. As well, it is highly unlikely that the alcohol was being elimination at a rate close to V_{max}, assuming a K_M for alcohol of 10 mg%. It would have been interesting to see what would have happened had the plasma alcohol levels been allowed to exceed 30 mg%. At such higher alcohol concentrations, would the rate of elimination approached V_{max} so as to offset the alcohol infusion rate or even exceed the rate of infusion? This of course would have produced either a plateau or a period of decline in the alcohol concentrations observed without the need to inject a bolus volume of alcohol as was done in the second half of the experiment. Clearly, had the experiment progressed to this level, and had either a plateau, or more dramatically, a decrease in plasma alcohol concentration occurred, then Eggleton would have been quite proper in concluding that the metabolic rate is a function of alcohol concentration, and it is doubtful that even today his conclusion would have been challenged. However, by injecting a bolus dose of ethanol into his cats during the second half of the experiment, he introduced two more variables. The first is the rate of distribution of the alcohol from the blood into the watery tissues. The second is the increased rate of excretion of alcohol via the breath and urine. Both the rate of distribution and the rate of excretion are dependent upon the concentration of alcohol in the plasma and therefore obey first order kinetics.

During the second half of the experiment, following the injection of the alcohol, the plasma alcohol levels rose very rapidly to in excess of 320 mg%. It would appear that Eggleton only waited about 1 hour between the end of the injection of the ethanol and what he perceived as the beginning of the elimination phase. Following this very rapid rise in the alcohol concentration, a period of distribution of this injected alcohol from the blood into the surrounding tissues would occur. This would in itself produce a rapid decline in the plasma alcohol concentration for some period of time until DE had been achieved. The rate of decline due to distribution would be dependent upon the relative difference in the alcohol concentration in the plasma and the surrounding watery tissues.

Linquist and Wolthers[115] reasoned that with human blood alcohol concentrations less than 50 mg%, the percentage of alcohol excreted in the breath and urine was less than 3%. However, with a six fold increase in the blood alcohol concentration (to 300 mg%), the percentage excreted increased five fold to 15%.

Separate from any concentration-dependent increase in metabolism, the rates of distribution and excretion of alcohol contribute to and explain in part the increase in the rate of plasma alcohol decline observed by Eggleton during the second half of his experiment. Because of this, it is improper to compare the change in the plasma alcohol concentration during this second half of the experiment to that of the first half and conclude that the change in the metabolic rate observed was due to a change in the concomitant alcohol concentration..

Clearly, it is absolutely necessary to define where DE has occurred for each subject tested prior to beginning measurements for alcohol elimination rates. As well, it is important when comparing the day to day intra-subject change in elimination rates that the contribution of excretion be kept relatively constant. For this thesis that was accomplished by standardizing the dose of alcohol given. For those such as Nagoshi and Wilson, and Wagner and Patel who did not define where the elimination phase actually begins but rather chose some arbitrary point, their results must be viewed with the gravest of suspicion.

A protocol for ensuring that a subject has achieved DE before measuring for $ß_{60}$ was introduced in Chapter 2 (see Figure 21 and Table 19). The technique is based upon the use of computerized multiple linear regression analyses which includes visualization of the proposed distribution/elimination phase of the alcohol concentration profile. This method has a number of advantages.

1. The technique of linear regression analysis is statistically quite simple.
2. Anyone with a primary understanding of computer basic programming can write the software.
3. It allows for a standardized protocol.
4. The technique provides an objective means of fitting the best line to the elimination data points.
5. It allows for reproducibility of results by others.
6. The post absorptive distribution/elimination phase (α phase) becomes visually distinct from the elimination phase (ß phase).
7. The user can see where the ß phase is in the context of the α phase, and therefore where DE is most likely to have occurred.

The present data showed that there was no significant gender difference in the time required to reach DE following the end of consumption (Figure 22).

The relationship between reaching DE and the time required to consume the alcohol is shown in Figure 23. The rate of absorption

of alcohol from the stomach into the blood, and the rate of distribution of the alcohol into the tissues both follow first order kinetics, that is, they are concentration-dependent. Generally, the more rapidly one consumes alcohol, the higher the concentration of alcohol there will be in the stomach for absorption, and the more rapidly the alcohol will be absorbed per unit of time. From this it follows that the more rapidly the alcohol is absorbed into the blood, the higher the venous blood alcohol concentration is going to be because the relative time required for distribution is limited. This in turn would be expected to increase the rate of distribution of alcohol from the venous blood throughout the body. Therefore, it would seem reasonable that the faster a subject consumes his dose of ethanol, the more quickly distribution will be complete and the shorter the time to DE. Figure 23 shows that indeed there was a positive correlation, albeit very low, between how quickly the alcohol was consumed by the subjects and the time required to reach DE ($r^2 = 0.2580$, $P < .001$).

The present data (Table 21.) shows that the time required to achieve DE is independent of the dose of alcohol consumed ($r^2 = 0.0603$, $P > .05$). This statistic is probably of limited value in light of the rather narrow range of doses administered. The range of doses for males was 0.454 to 1.064 ml 95% (v/v) alcohol/kg, and for females 0.464 to .922 ml/kg.

Elimination Rates

The rate of alcohol elimination ranges from 6 to 24 mg/ 210 L of breath per hour. For males the average rate is 13.5 ± 2.5 mg/ 210 L of breath per hour. This rate is significantly lower than that for females which is 15.2 ± 3.26 mg/210 L/ hour. Although the difference is statistically significant, there is not a strong gender difference ($r_{PB} = 0.172$). Since these rates reflect the concomitant rate of decrease in the blood, they are equivalent to a blood alcohol elimination rate for males of 13.5 mg/ 100 ml blood per hour (13.5 mg%/hour) and for females of 15.2 mg%/hour. Dubowski[116] reported a mean elimination rate for 134 males tested of 14.94 ± 4.50 mg/230 L/hour, with a range of 5.9 to 27.9. Converting these results to units of mg/210 L/hr, Dubowski's results are equivalent to 13.6 ± 4.1 mg/210 L/hour, with a range of 5.4 to 25.5 mg/210 L/hour. There is very close agreement between the present results and those of Dubowski. However, as stated in the previous section,

these results are not consistent with those of Nagoshi and Wilson[117], and, in fact, are significantly lower.

The present results are also significantly lower (t = -2.44, $P < .015$, r_{PB} = 0.091) than those reported by Winek and Murphy[118] which showed a mean elimination rate of 15.2 ± 3.15 mg%/hour. Their protocol does not indicate the gender of the subjects tested, but refers to them only as "individuals". What is unique about Winek and Murphy's research is that they published the correlation coefficients for their "zero order elimination rate" linear regression analyses. However, their mean correlation coefficient of -0.9293 ± 0.052 was significantly lower (t = -21.70, $P < .001$, r_{PB} = 0.79) than those of the Group I subjects (males and females) which was -0.9908 ± 0.009). The majour error that Winek and Murphy made was assuming that the peak blood alcohol concentration marked the beginning of the ß phase. As mentioned earlier in relation to the Nagoshi and Wilson research, such an error tends to hybridize both the α and the ß phase, resulting in elevated values for $ß_{60}$. This may explain Winek and Murphy's higher average $ß_{60}$ and significantly lower correlation coefficients when compared to the present data.

The hourly rate of elimination for females was found to be statistically higher than for the male subjects (r_{PB} = 0.172), which is inconsistent with Widmark's[119] observations. Since Widmark's time there has been, however, much controversy about differences in the rates of elimination for males and females. Rachamin *et al.*[120] observed that the rate of elimination in immature male rats was not significantly different from the mature female rats, but that there was a significant difference between the rate for mature male rats and female rats. He reasoned the elevated levels of testosterone in the mature male rats were responsible for suppressing the alcoholic metabolic rate in comparison to the female rats and the immature rats.

Watson *et al*[121] argued that any difference is due to females having a lower volume of distribution (Widmark's r factor) than males. Since women have proportionately more body fat than males, they have proportionately less body water for the alcohol to be distributed in, and therefore, females will gain a higher peak blood alcohol concentration per dose of alcohol than males of equal stature. This higher alcohol concentration is therefore supposed to result in a faster rate of elimination in the females. The present data showed that there was a negative correlation of -0.4134 and -0.3872 between Widmark's r factor and $ß_{60}$ in the Group I

Subjects (Table 21) and the Group II Subjects (Table 25) respectively.

Frezza *et al.*[122] argued that the inter-gender difference in the elimination rate is not a function of the volume of distribution, but of the bioavailability of alcohol to the circulating blood. When they administered alcohol by intravenous infusion, rather than orally, their female and male subjects eliminated ethanol at similar rates of 90.9 ± 6 mg/kg/hour and 93.7 ± 5.0 mg/kg/hour respectively. What Freeza and his co-workers argue is that because men experience a greater degree of first-pass metabolism of alcohol through the gastric mucosa than women, men obtain lower blood alcohol concentrations than women given the same weight corrected dose of alcohol. When the effects of first-pass metabolism are avoided by administering the alcohol intravenously, the inter-gender difference in the rate of elimination is nullified. This, in spite of the fact that the women continue to have a significantly lower volume of distribution than their male counterparts (0.686 ± 6 L/kg for females versus 0.767 ± 4 L/kg for males). These values for the volume of distribution for females and males are similar to the present data of 0.65 ± 0.11 L/kg for females and 0.75 ± 0.09 L/kg for the males.

Another area of controversy concerning the inter-gender differences in elimination rates concerns the influence of sex hormones on alcohol metabolism. Jones and Jones[123] demonstrated that those female subjects taking oral contraceptives had a significantly lower rate of alcohol elimination (105 mg/kg/hour) than those not taking oral contraceptives (121 mg/kg/hour). This difference in the elimination rate persisted during the menstrual, intermenstrual and premenstrual phases of the menstrual cycle. As well, Jones and Jones showed that those women taking oral contraceptives experienced lower peak blood alcohol concentrations than the women who were not taking oral contraceptives. However, there was no significant difference between those taking oral contraceptives and those not in the time to reach the peak blood alcohol concentration, the rate of absorption (peak blood alcohol concentration divided by the time to reach the peak) nor the slope of the ascending alcohol concentration.

Unfortunately, the Jones and Jones breath testing protocol is fraught with a number of technological errors which brings into doubt the validity of their conclusions.

Immediately after the Jones and Jones subjects had consumed their alcohol, the subjects rinsed out their mouths with water "... *to clear it of residual ethanol.*"[124] One of the major disadvantages of breath testing is that alcohol persists in the oral cavity for about 15 minutes after the last consumption. During breath collection, this mouth alcohol combines with the alcohol from the lungs, resulting in falsely high readings. Consequently, it is almost impossible to track the changes in the person's alcohol concentration immediately after drinking, something that is desirable if one is attempting to measure rates of absorption and the time to the peak alcohol concentration. Attempts to rinse out this residual mouth alcohol with water seldom succeeds for two reasons. The first reason deals with the temperature of the water being used as a rinse. If cool water is used as a rinse, it will decrease the normal temperature of the subject's mouth which in turn cools the breath sample. The denser breath then has a higher alcohol content for the same volume which results in false high readings. Conversely, if the water is warmer than the subject's normal mouth temperature, the oral cavity is abnormally warmed, causing the breath to have a lower alcohol concentration and therefore resulting in falsely lower readings. The second reason is that for unknown reasons, rinsing the mouth seldom succeeds in eliminating the alcohol from the oral cavity following consumption.[125] Therefore, the results of breath testing during the fifteen minutes following consumption will not provide an accurate reflection of the changes in the corresponding blood alcohol concentration during this period of time.[126,127]

The second difficulty faced by Jones and Jones is in establishing when the blood alcohol concentration of their subjects returned to zero. They divided the total dose of alcohol (mg/kg) that their subjects consumed by the time required to return to a zero blood alcohol concentration (hours) to produce a statistic reflecting the elimination rate of alcohol from the whole body (mg/kg/hour). The breath analyzer that they used was an Omicron Intoxilyzer Model 4011 which utilizes infrared spectrophotometry. Characteristically, such devices record a zero reading when the breath alcohol concentration becomes less than 10 mg/210 L of breath. Therefore, an accurate measure of when the alcohol concentration actually returned to zero is seldom possible. As well, it is important to note that below breath alcohol concentrations of 20 mg/210 L breath, the concentration approaches a zero value asymptotically which also makes it difficult to accurately determine at what time the alcohol concentration returned to zero.

While Jones and Jones found that oral contraceptives slowed the rate of alcohol elimination in females, Cole-Harding and Wilson[128] found that neither oral contraceptives nor the phase of the menstrual cycle had an effect on the hourly rate of ethanol elimination. Cole-Harding and Wilson also made use of breath testing to monitor changes in blood alcohol concentrations. In doing so, they made similar assumptive errors to Jones and Jones[129] concerning rinsing out the mouth with water and attempting to determine the time when the alcohol concentration returned to zero. In addition, Cole-Harding and Wilson also confounded their attempts to measure the decline in the breath alcohol concentration by providing a lunch to their subjects after the last alcohol had been consumed[130]. This lunch could have a non-reproducible effect, not only on the absorption of the alcohol from the stomach into the portal system, but could have also resulted in a transient decrease in the rate of alcohol elimination[131] confounding their attempts to measure the pseudo-linear rate of decline of the breath alcohol concentration.

Clearly, until a standardized protocol is established for accurately and reproducibly measuring the rate of alcohol elimination in humans, controversy will persist concerning what is responsible for the inter-gender difference in human elimination rates.

A positive correlation was shown to exist between β_{60} and the age of the Group I subjects (Figure 26 and Table 21), contrary to the earlier findings of Vestel *et. al.*[132] Possibly due to the smaller sample size, such a correlation did not exist for the Group II subjects.

Figure 27 shows that there was no correlation between the weight of the male or the female subjects and β_{60}. There was, however, a negative correlation between β_{60} and the height of males, but not the height of females. When the height of the subjects is divided by their weight to produce the ponderal index (PI), there is no correlation between the elimination rate and PI ($P > .05$) for either males or females, whether they be in Group I or in Group II.

Intrasubject Variability in Elimination Rates

While there is a wide range of elimination rates between individuals (6 to 24 mg/ 210 L/ hour), day to day rates of elimination for an individual are very robust. For the 29 male and female subjects tested on two different days, there were no significant differences in their average rates of elimination. The mean day to day difference

was 0.05 ± 1.8 mg/ 210 L/ hour and was independent of the interval of time between testing (Table 25). The day to day variation ranged from -3.8 to 3.2 mg/ 210 L/ hour, with the difference not exceeding ± 2 mg/210 L/hour 72.4% of the time.

Three factors are identified as having an effect on the day to day difference in individual elimination rates. The higher one's rate of elimination, the greater will be the difference from day to day in the rates. Statistically, this makes sense. The further a subject's rate is from the norm, the greater the change it must undergo to approach normality.

The second factor is the time required to reach DE. As the time required to reach DE increases, the day to day difference diminishes. Since the elimination rate is concentration-dependent, the longer it takes to achieve DE, the longer the period of time for the alcohol to be eliminated, and, therefore, a lower resulting alcohol concentration when DE is achieved.

The third factor is the volume of distribution (Widmark's *r* factor). The lower the value for *r*, the greater the day to day difference in the rate. This is not too surprising especially when one considers that women have proportionately less body water than males, and hence a lower *r* factor. Women also have significantly higher elimination rates. The lower the value of *r*, the higher the blood alcohol concentration for a given dose. The higher the blood alcohol concentration, the higher the elimination rate. As mentioned, there is a positive correlation between the $ß_{60}$ and the day to day difference. Therefore the higher the elimination rate the higher the day to day difference.

The test-retest correlation for the elimination rates is shown in Figure 32 and was determined to be 0.7122 ($P < .001$). This is a significantly higher repeatability value than the zero value quoted by Nagoshi and Wilson [133]. In terms of their own data, they do, however, concede that

> "... there is less than 20% difference in $ß_{60}$ values between the test and the retest for most of the cases."[134]

A day to day difference of 20% would be equivalent to 2.6 mg/210 L/hour, which is consistent with the present data.

Clearly, elimination kinetics for ethanol cannot be described in purely mathematical terms. The contribution of excretion, however slight, follows first order kinetics whereas metabolism in all probability follows the saturation kinetics of Michaelis-Menten.

Simply assuming that elimination follows zero order kinetics belies the rate of change in the blood alcohol concentration below 20 mg%. Attempts to describe human alcohol elimination in terms of purely classical kinetic theory is fraught with frustration and destined for failure. Greater success may be possible with a more *avant-garde* approach which would unleash the extensive technology of computerized pattern recognition: a pattern of alcohol concentration changes with time.

Conclusion

Pharmacologists are often asked in both criminal and civil courts to predict a blood alcohol concentration at a particular period of time based upon a person's weight, age, height, pattern of drinking and volume of consumption. In the civil courts the test is one of what is more likely than not. In the criminal courts, the test is more severe. The Crown in proving its case must prove it beyond any reasonable doubt. In defence, the accused must introduce a reasonable doubt.

This thesis has shown that the most probable elimination rate for males is 14 mg%/hour, slightly slower than the rate for females of 15 mg%/hour. While the rate is dependent upon the coexisting blood alcohol concentration, and decreases as the concentration of alcohol in the blood decreases, the difference is slight. On page 83 of this thesis, Forrest is described as showing that the rate only changes by 2.6 mg% per hour over a blood alcohol concentration range of 50 to 200 mg%.

Often of concern to the Courts is the change in elimination rates that an individual can experience from one day to the next. While there is admittedly a day to day variation, it is not significant. Over 70% of the time the difference will not exceed more than 2 mg/210 L/hour from day to day, which is independent of the interval of time between the days in question. Table 27 shows that 80% of the time, predictions about a person's probable blood alcohol concentration, based upon their ACI, weight, β_{60}, the amount of ethanol consumed, and the timing of that consumption, are no more than 10% higher than the actual alcohol concentration.

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11 *Ibid.*, 43-48

12 Widmark, E.M.P. Principles and Applications of Medicolegal Ethanol Determination, Translation of Die theoretischen Grundlagen and die praktische Verwendbarkeit der gerichlich-medizinischen Alkoholbestimmung, (Biomedical Publications, Davis California, 1981)

13 Nagoshi C.T. and Wilson, J.R. (1989) Long-Term

[14] Widmark used ‰ (*per mille*) to express his blood ethanol concentrations . Therefore, a 1 ‰ ethanol concentration was equivalent to 1 mg ethanol in 1 gram of blood. To convert these units to mg/100 ml (mg%), I have multiplied Widmark's values by 105.5 which accounts for the average specific gravity of blood and the decimal place movement.

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[16] *Ibid.*, 70

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Appendix

Appendix A. Subject Information Sheet

ALCOHOL PHARMACOKINETIC TESTING by our laboratories is a non-invasive, very relaxing procedure designed to determine a particular individual's rate of alcohol elimination from his body, and the blood alcohol concentration that that individual achieves for each volume of alcohol consumed.

The laboratory begins with a brief explanation of what will occur during the next four to five hours of testing. Physical characteristics such as body weight, age, sex, and height are recorded. A short "medical interview" is conducted to determine if the subject is currently on any prescribed drugs, is a smoker, presently on a diet, has had any diseases of the central nervous system such as epilepsy, has ever suffered from alcoholism, has any allergies, has had any liver disorders, the number of hours since the last meal, and if a female subject whether or not she is on the "pill". An initial breath test will then be taken to ensure a zero blood alcohol concentration exists.

NOTE: It is important to note that subjects with an initial blood alcohol concentration greater than zero milligrams of alcohol per 100 millilitres of blood (>0 mg%) will not be allowed to complete the testing, and will be asked to leave. When this occurs, the laboratory fee is forfeited.

Upon completion of the interview, each subject will be advised of the amount of alcohol (mixed with orange juice) he will be required to drink. Generally, it is an amount of alcohol required to raise the blood alcohol concentration to between 80 and 100 mg%. Each subject is then required to read and sign a *Waiver and Consent to the Administration of Alcohol and the Taking and Analysis of Breath Samples.*

The subject is then given an alcohol cocktail of absolute alcohol mixed with orange juice. Generally this is consumed within 15 minutes. During the next four to five hours, in the comfort of a living room setting, approximately thirty breath samples will be collected from the subject and analyzed using the latest in breath alcohol testing technology. The interval between sampling ranges from 2 minutes to 8 minutes. The subject will be free to leave once the breath alcohol concentration returns to zero.

In Preparation For Pharmacokinetic Testing, the subject must:

1. refrain from eating solid foods for a period of 5 hours before the laboratory appointment,
2. abstain from the consumption of any alcohol for a period of 24 hours before the laboratory appointment,
3. abstain from at least 48 hours from the use of any drugs which, combined with alcohol, could have a serious effect on him, and

4. arrive at the laboratory no earlier than 15 minutes before the time of the appointment, and no later that the time of the appointment.

SPECIAL CIRCUMSTANCES

1. Subjects who have a medical history of alcoholism will not normally be allowed to undergo testing by our laboratories since consumption of alcohol during the lab procedure is required. Only under very special circumstances, and with the written approval of a medical practitioner or alcohol counselor will such testing be conducted by our laboratory.
2. Since alcohol may precipitate an epileptic seizure, our laboratory will not conduct alcohol testing of persons with a medical history of epilepsy unless written approval is first received by our laboratory directly from the subject's doctor.
3. Since alcohol has been linked to the development of Fetal Alcohol Syndrome, our laboratories will not test women who are pregnant. It will be your responsibility to ensure that you are not pregnant before attending our lab for testing.

If you have any questions, please do not hesitate to give us a call at (403) 467-2077.

LOOKING FORWARD TO MEETING YOU - BRUCE D. MILLER

Appendix B. Drinking Subject Interviews

1. Date:
2. Subject Number:
3. Sex: M F
4. Age: Years
5. Weight: Lbs.
6. Height (Feet-Inches):
7. Medical Condition:

a. Presently on any drugs? Y N
b. Smoker? Y N
c. Ever any problems with your liver? Y N
d. On any diet? Y N
e. Any diseases of the CNS? Y N
f. Any allergies? Y N
g. Ever suffered from/treated for alcoholism? Y N
h. Ever been treated for diabetes? Y N
i. If female, on the pill? Y N

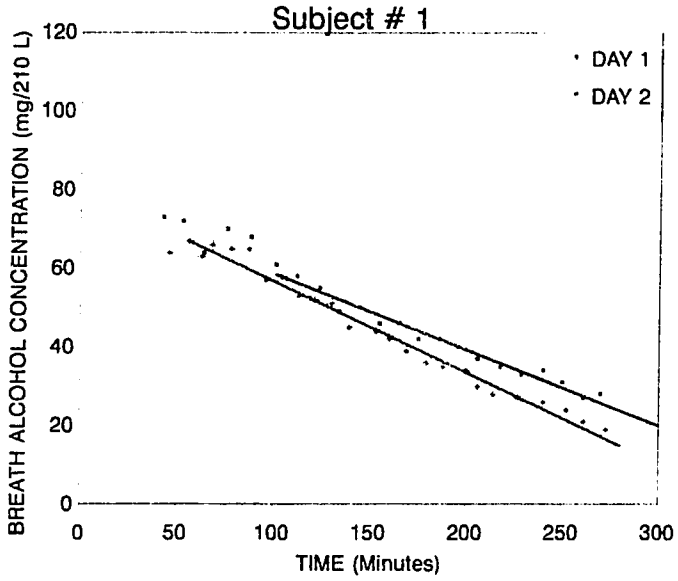
8. Hours since last meal
9. Initial BrAC : @

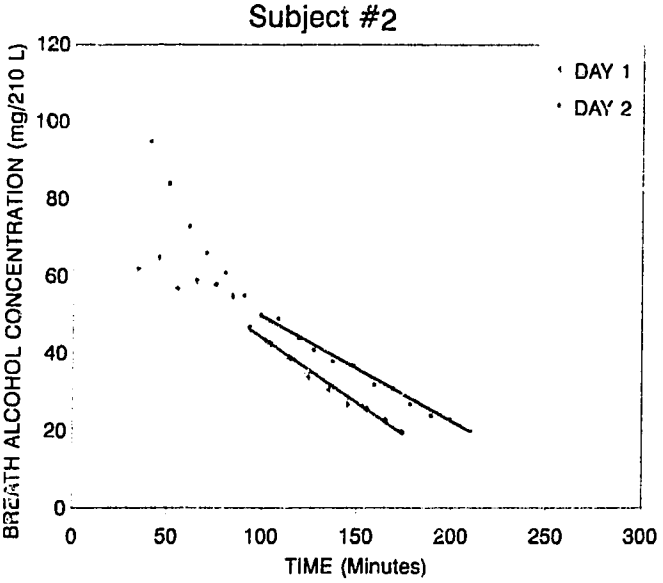
ABS = .085 x LBS. x 0.35 = OZ. LIQUOR x 40%(V/V) = OZ. EtOH
= OZ. EtOH/0.035 195 080 OZ./ML
= ML EtOH

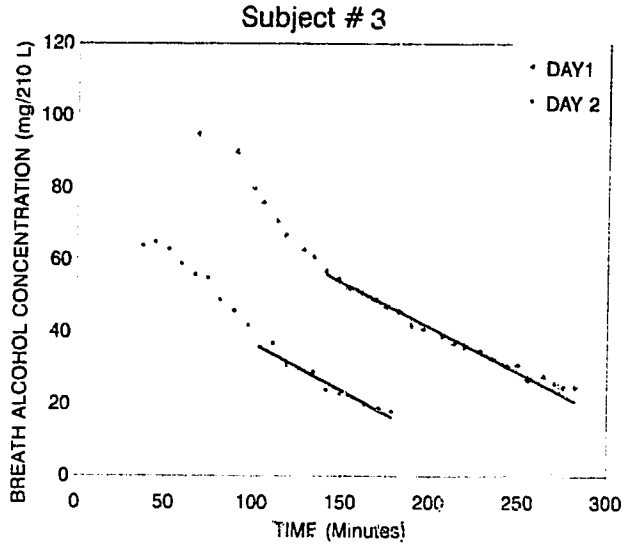
- Begins drinking (Amount)
- Finishes

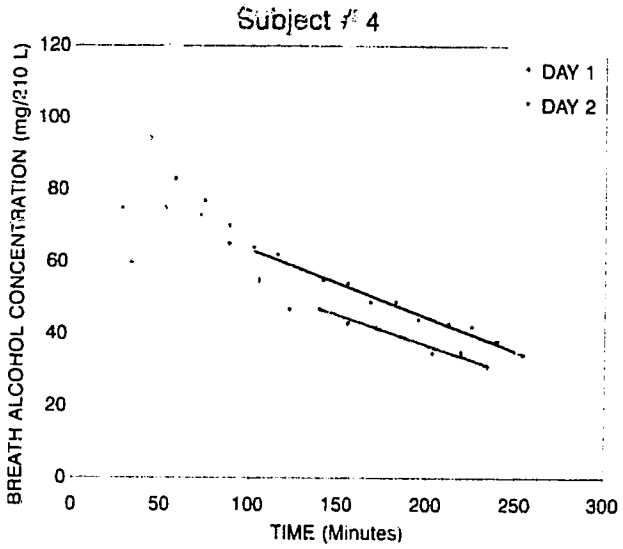
	TIME	BrAC	MIN		TIME	BrAC	MIN		TIME	BrAC	MIN
1				11				21			
2				12				22			
3				13				23			
4				14				24			
5				15				25			
6				16				26			
7				17				27			
8				18				28			
9				19				29			
10				20				30			

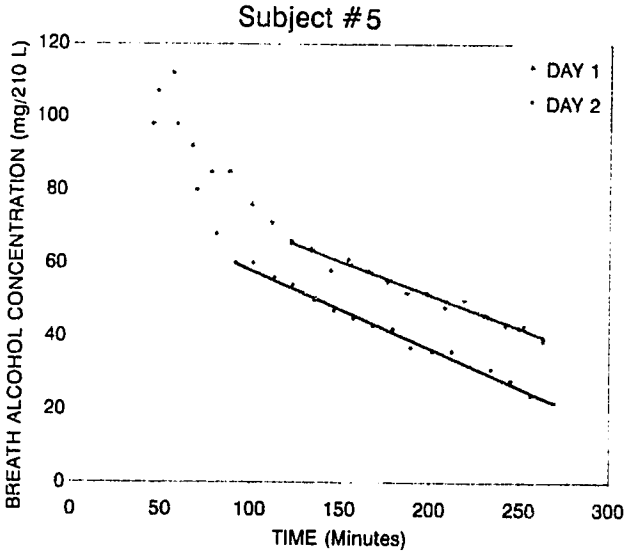
Appendix C. Group II Alcohol Concentration Profiles

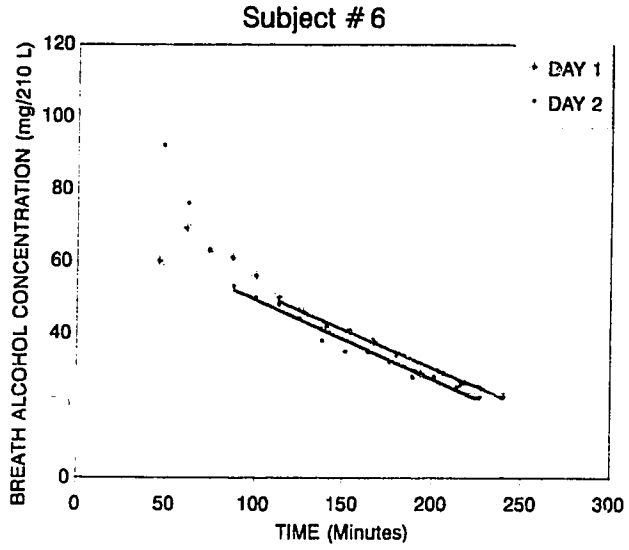


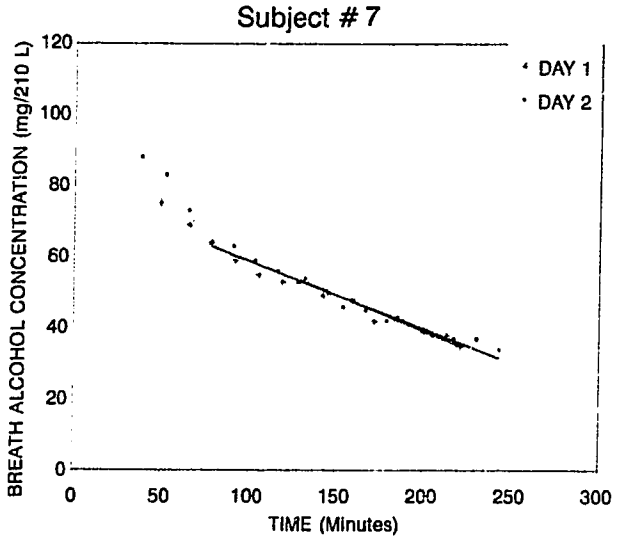


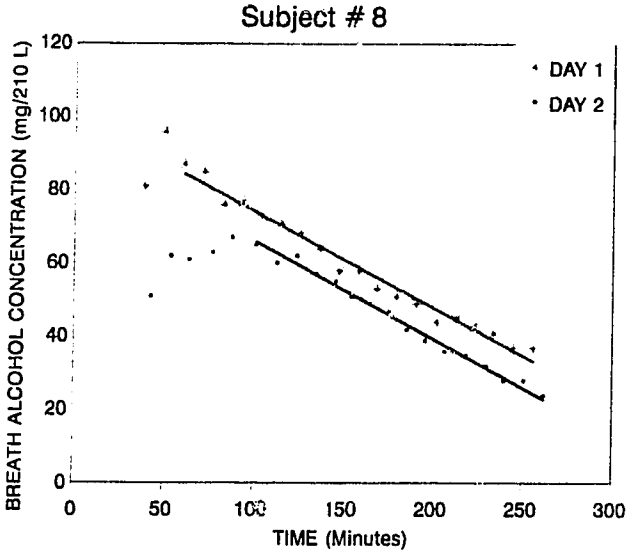

Subject # 3
BREATH ALCOHOL CONCENTRATION (mg/210 L)
120
100
80
60
40
20
0
0
50
100
150
200
250
300
TIME (Minutes)
DAY1
DAY 2

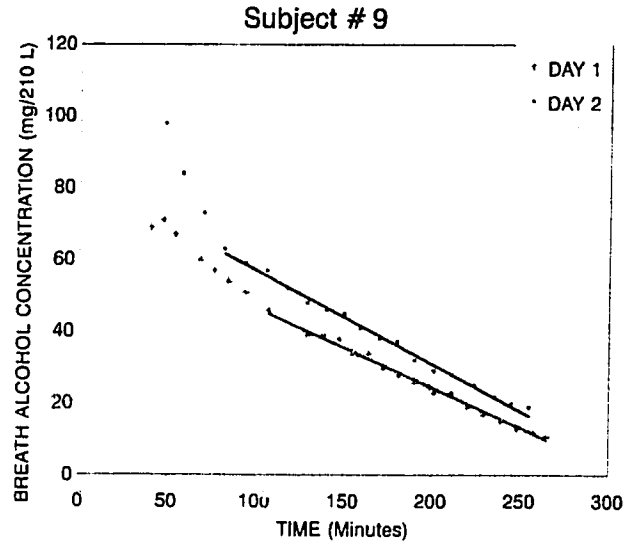

Subject # 4
BREATH ALCOHOL CONCENTRATION (mg/210 L)
120
100
80
60
40
20
0
0
50
100
150
200
250
300
TIME (Minutes)
DAY 1
DAY 2

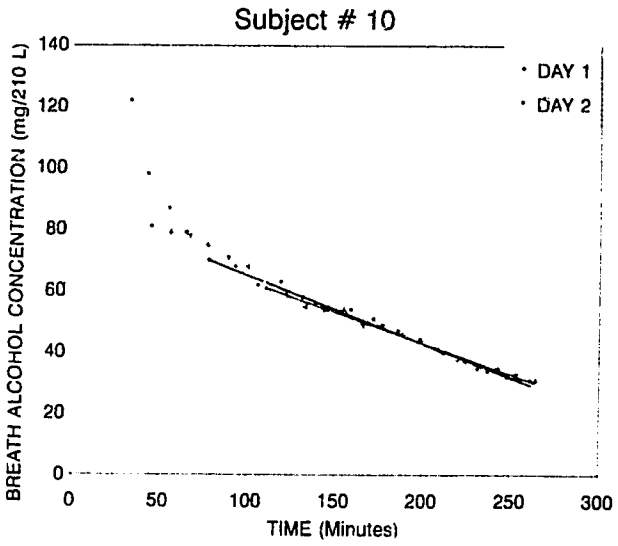

Subject #5
BREATH ALCOHOL CONCENTRATION (mg/210 L)
120
100
80
60
40
20
0
0
50
100
150
200
250
300
TIME (Minutes)
DAY 1
DAY 2

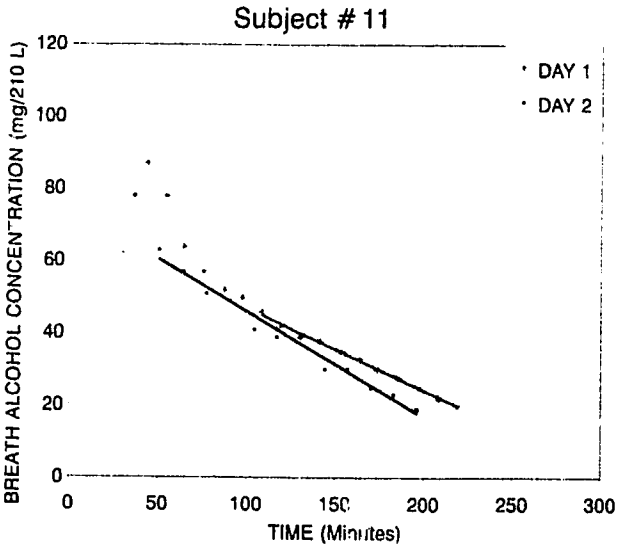

Subject # 6
BREATH ALCOHOL CONCENTRATION (mg/210 L)
120
100
80
60
40
0
0
50
100
150
200
250
300
TIME (Minutes)
DAY 1
DAY 2

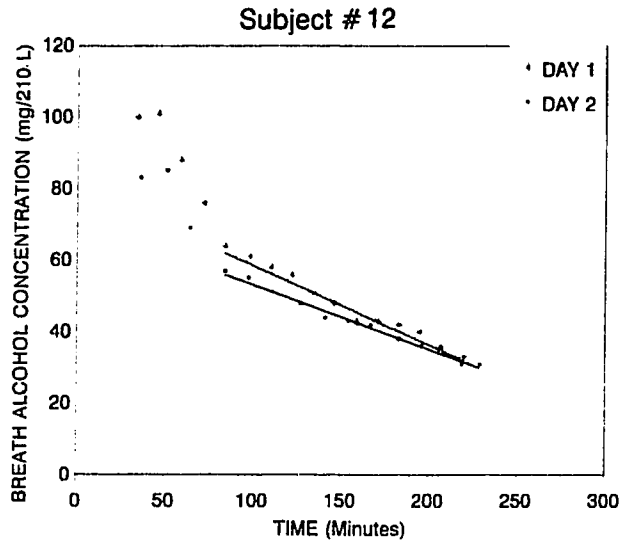

Subject # 7
BREATH ALCOHOL CONCENTRATION (mg/210 L)
120
100
80
60
40
20
0
0
50
100
150
200
250
300
TIME (Minutes)
DAY 1
DAY 2

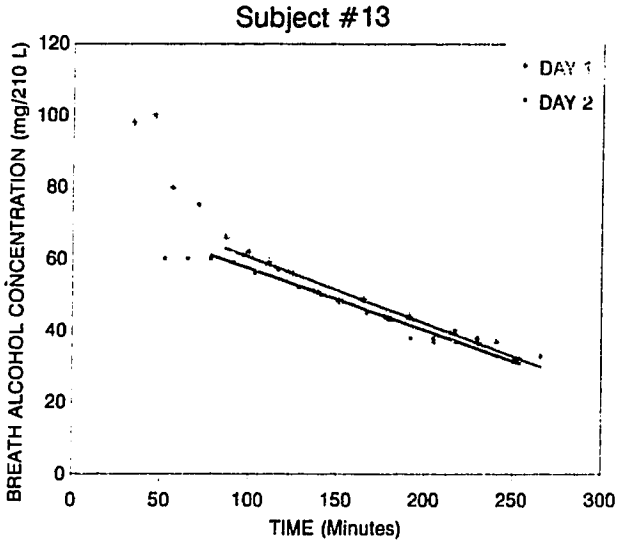

Subject # 8
BREATH ALCOHOL CONCENTRATION (mg/210 L)
120
100
80
60
40
20
0
0
50
100
150
200
250
300
TIME (Minutes)
DAY 1
DAY 2

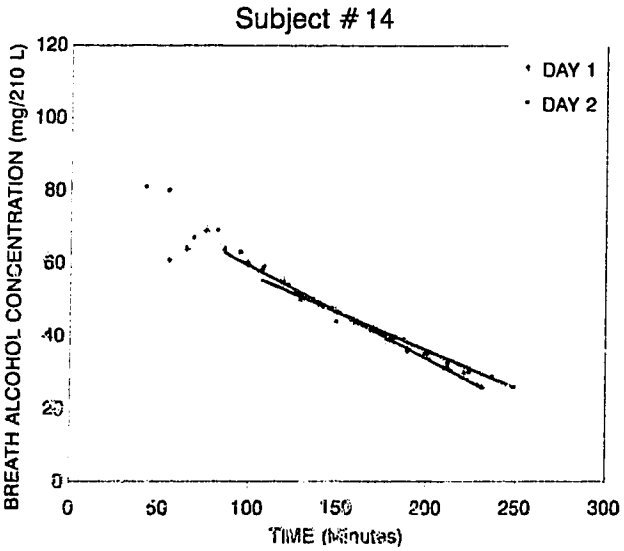

Subject # 9
BREATH ALCOHOL CONCENTRATION (mg/210 L)
120
100
80
60
40
20
0
0
50
100
150
200
250
300
TIME (Minutes)
DAY 1
DAY 2

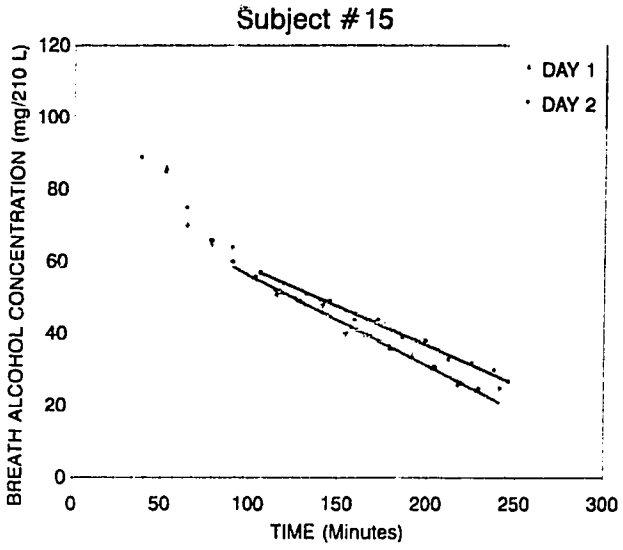

Subject # 10
BREATH ALCOHOL CONCENTRATION (mg/210 L)
140
120
100
80
60
40
20
0
0
50
100
150
200
250
300
TIME (Minutes)
DAY 1
DAY 2

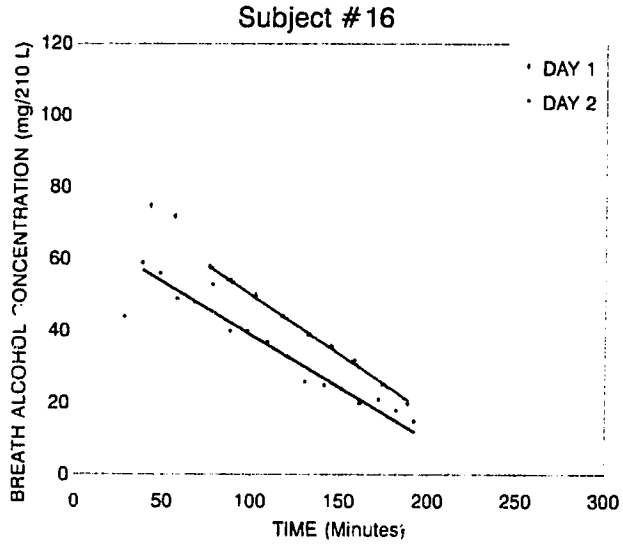

Subject # 11
BREATH ALCOHOL CONCENTRATION (mg/210 L)
120
100
80
60
40
20
0
0
50
100
150
200
250
300
TIME (Minutes)
DAY 1
DAY 2

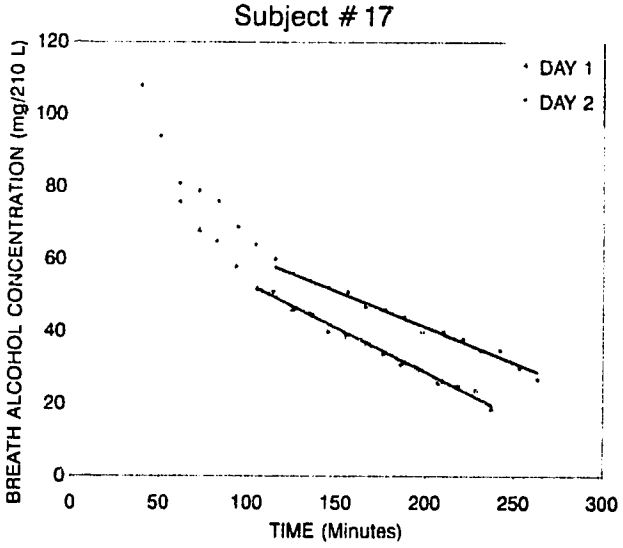

Subject # 12
BREATH ALCOHOL CONCENTRATION (mg/210 L)
TIME (Minutes)
DAY 1
DAY 2
0
20
40
60
80
100
120
0
50
100
150
200
250
300

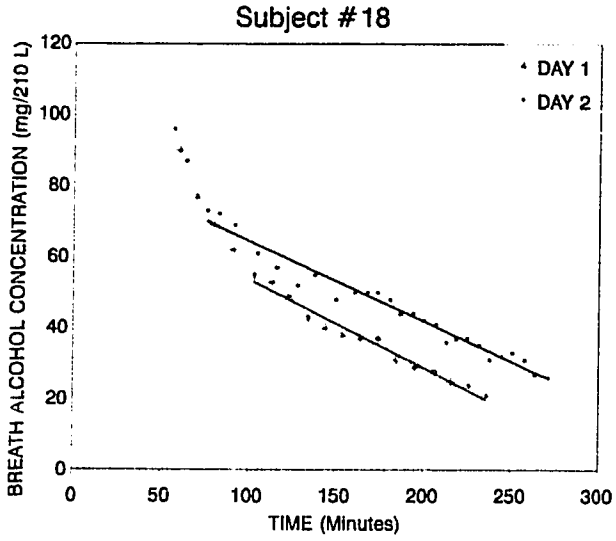

Subject #13
BREATH ALCOHOL CONCENTRATION (mg/210 L)
TIME (Minutes)
DAY 1
DAY 2
0
20
40
60
80
100
120
0
50
100
150
200
250
300

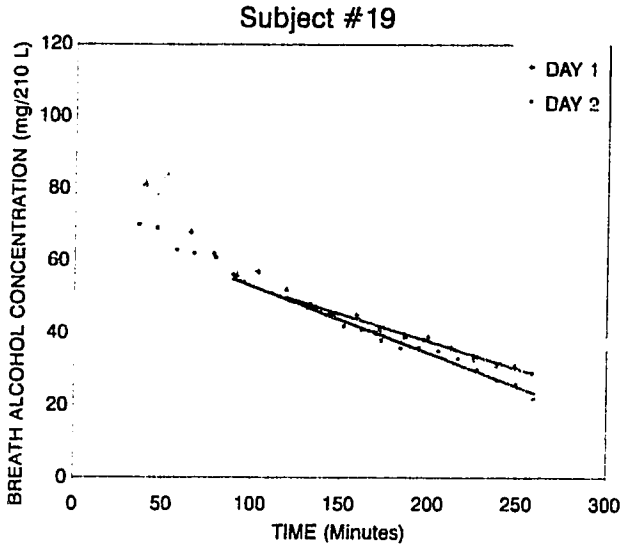

Subject # 14
BREATH ALCOHOL CONCENTRATION (mg/210 L)
TIME (Minutes)
DAY 1
DAY 2
0
20
40
60
80
100
120
0
50
100
150
200
250
300

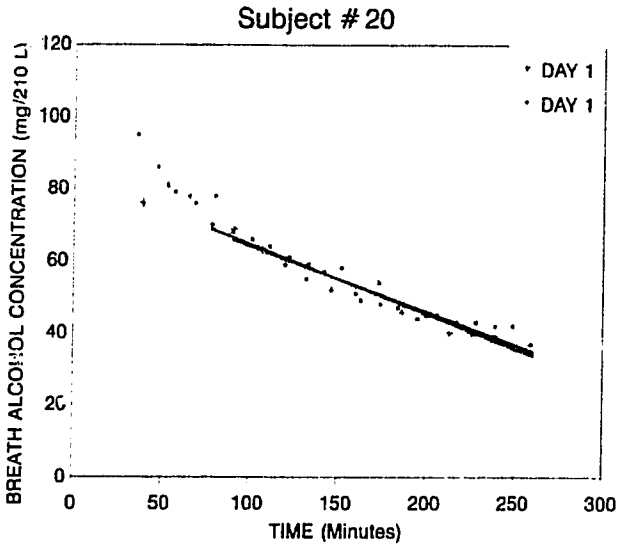

Subject #15
BREATH ALCOHOL CONCENTRATION (mg/210 L)
120
100
80
60
40
20
0
0
50
100
150
200
250
300
TIME (Minutes)
DAY 1
DAY 2

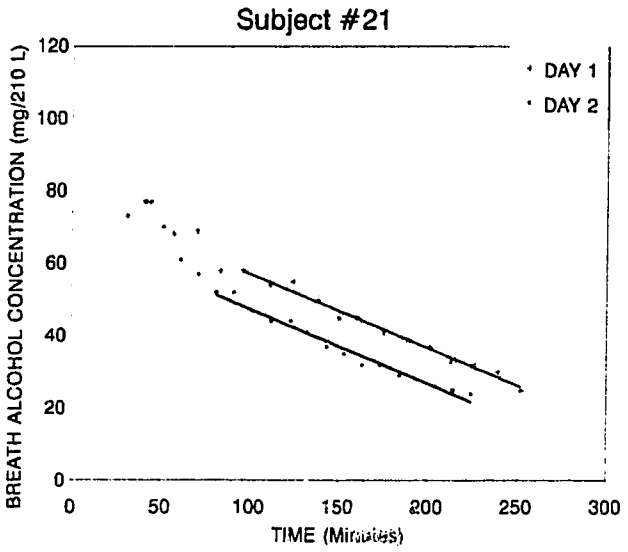

Subject #16
BREATH ALCOHOL CONCENTRATION (mg/210 L)
120
100
80
60
40
20
0
0
50
100
150
200
250
300
TIME (Minutes)
DAY 1
DAY 2

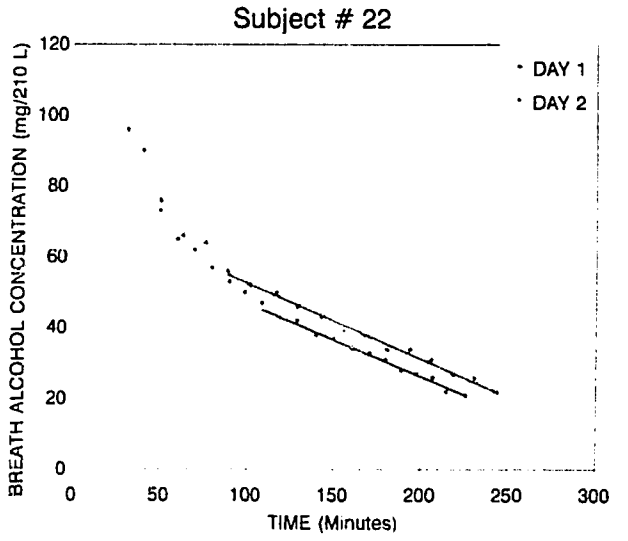

Subject #17
BREATH ALCOHOL CONCENTRATION (mg/210 L)
120
100
80
60
40
20
0
0
50
100
150
200
250
300
TIME (Minutes)
DAY 1
DAY 2

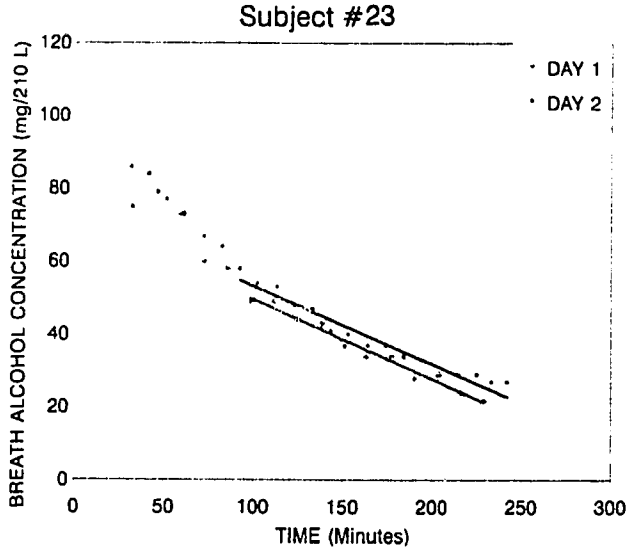

Subject #18
BREATH ALCOHOL CONCENTRATION (mg/210 L)
TIME (Minutes)
DAY 1
DAY 2
0
20
40
60
80
100
120
0
50
100
150
200
250
300

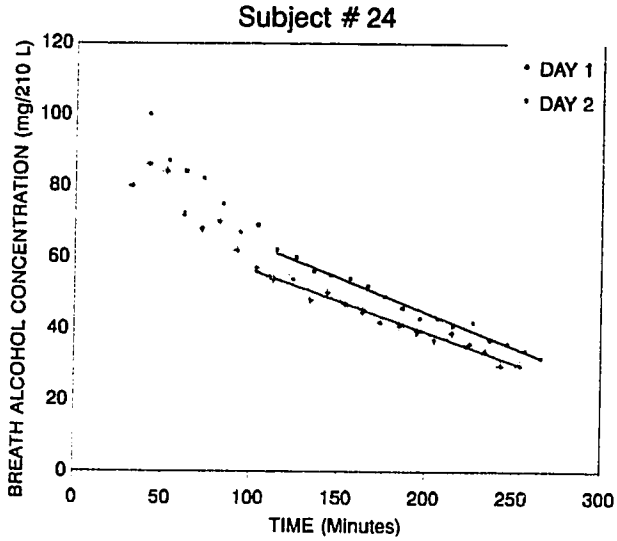

Subject #19
BREATH ALCOHOL CONCENTRATION (mg/210 L)
TIME (Minutes)
DAY 1
DAY 2
0
20
40
60
80
100
120
0
50
100
150
200
250
300

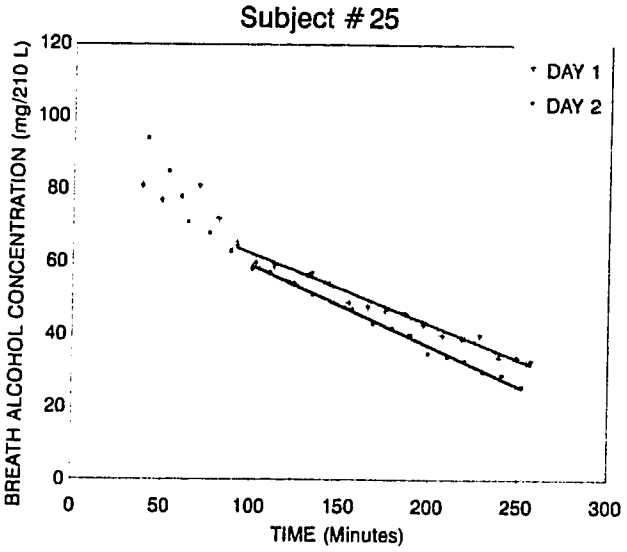

Subject #20
BREATH ALCOHOL CONCENTRATION (mg/210 L)
TIME (Minutes)
DAY 1
DAY 1
0
20
40
60
80
100
120
0
50
100
150
200
250
300

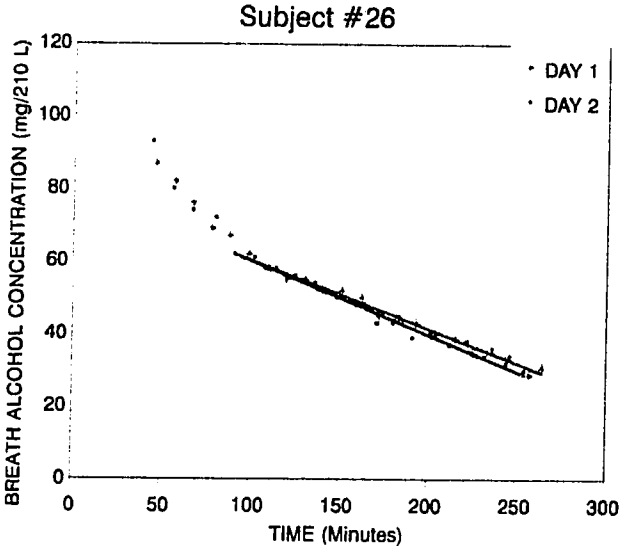

Subject #21
BREATH ALCOHOL CONCENTRATION (mg/210 L)
120
100
80
60
40
20
0
0
50
100
150
200
250
300
TIME (Minutes)
DAY 1
DAY 2

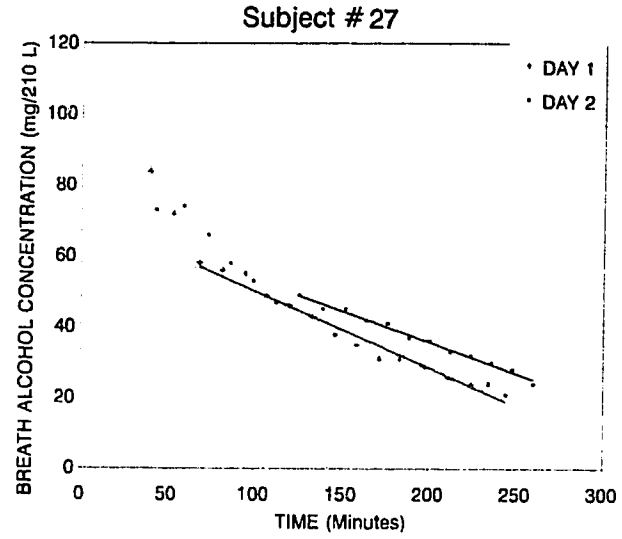

Subject # 22
BREATH ALCOHOL CONCENTRATION (mg/210 L)
120
100
80
60
40
20
0
0
50
100
150
200
250
300
TIME (Minutes)
DAY 1
DAY 2

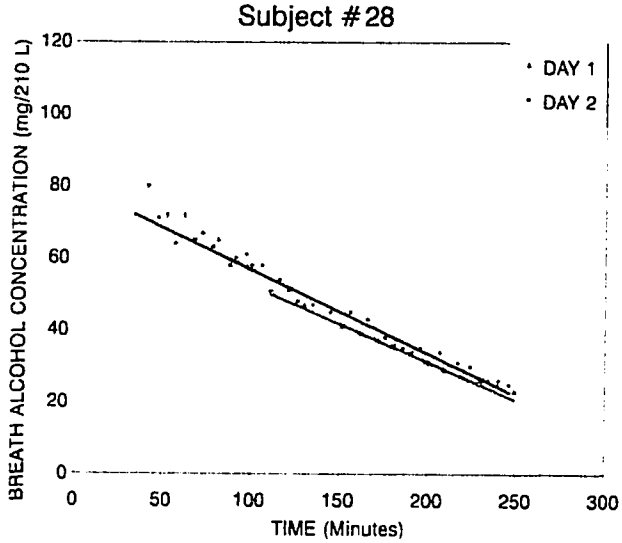

Subject #23
BREATH ALCOHOL CONCENTRATION (mg/210 L)
120
100
80
60
40
20
0
0
50
100
150
200
250
300
TIME (Minutes)
DAY 1
DAY 2

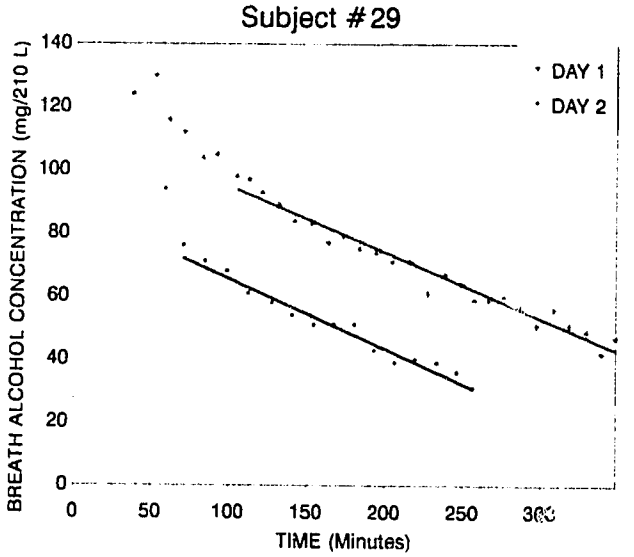

Subject # 24
BREATH ALCOHOL CONCENTRATION (mg/210 L)
120
100
80
60
40
20
0
0
50
100
150
200
250
300
TIME (Minutes)
DAY 1
DAY 2


Subject # 25
BREATH ALCOHOL CONCENTRATION (mg/210 L)
120
100
80
60
40
20
0
0
50
100
150
200
250
300
TIME (Minutes)
DAY 1
DAY 2


Subject #26
BREATH ALCOHOL CONCENTRATION (mg/210 L)
120
100
80
60
40
20
0
0
50
100
150
200
250
300
TIME (Minutes)
DAY 1
DAY 2


Subject # 27
BREATH ALCOHOL CONCENTRATION (mg/210 L)
120
100
80
60
40
20
0
0
50
100
150
200
250
300
TIME (Minutes)
DAY 1
DAY 2


Subject # 28
BREATH ALCOHOL CONCENTRATION (mg/210 L)
120
100
80
60
40
20
0
0
50
100
150
200
250
300
TIME (Minutes)
DAY 1
DAY 2


Subject # 29
BREATH ALCOHOL CONCENTRATION (mg/210 L)
140
120
100
80
60
40
20
0
0
50
100
150
200
250
300
TIME (Minutes)
DAY 1
DAY 2

Appendix D. Breath Testing Protocol

Prior to attendance at the laboratory, each subject was provided with the Subject Information Sheet (Appendix A.). Upon arrival at the laboratory, each subject was weighed, and given an initial breath test to ensure a zero residual alcohol concentration. The subjects were asked the questions contained on the Drinking Subject Interviews form (Appendix B.) and the responses duly recorded. Based upon the subject's weight, the dose of alcohol to be consumed was calculated and measured out using a 50 ml burette. The alcohol was mixed with reconstituted orange juice. Subjects tested between March 1994 and September 1987 had their breaths analyzed by a Gas Chromatographic Intoximeter, Mark IV (Intoximeters, Inc., St. Louis, MO.). Those subjects tested subsequent to April 1989, were tested with the Intoximeter 3000 (Intoximeters, Inc., St. Louis, MO.). There was no testing conducted between September 1987 and April 1989.

Both instruments had their calibration checked by aqueous alcohol standards. A stock solution of 77 ml ethanol made up to a volume of 1.0 L with distilled water was used to prepare the 500 ml standard alcohol solutions. Each ml of stock solution diluted with distilled water to a volume of 500 ml, generated a simulated alcohol breath sample of 10 mg/210 L breath when heated in an Alcoholic Breath Mark IA Simulator (Smith & Wesson, Springfield, Ill.) to 34°C. Once the thermometer in the Simulator indicated 34°C, breath was blown through the inlet tube at the top of the Simulator, directed down through a tube to the bottom of the Simulator, and dispersed out tiny holes bored along the terminal length of the tubing. The breath then escaped through an outlet tube on the side of the Simulator, through Tygon® tubing to the breath inlet tube of the breath testing instrument. The concentration of alcohol carried on the breath is a function of temperature and the concentration of alcohol in the standard alcohol solution. Ten ml of stock solution, diluted to 500 ml with distilled water, and heated to 34°C, simulates a BrAC of 100 mg/210 L.

The procedure for making up standards and the use of the Alcoholic Breath Simulator Mark IVA is described in the Breathalyzer® Model 900A instructional Manual (Smith & Wesson, 2100 Roosevelt Ave., Springfield, Mass. 01101).

Gas Chromatographic Intoximeter Mark IV

The Gas Chromatographic Intoximeter Mark IV (GCI) was developed by CalDetect, Inc., Richmond, California. It is designed to directly analyze the alcohol content of breath samples. The unit is housed in a single chassis containing the breath sampling valve, the column, the flame ionization detector (FID), the strip chart recorder and the digital readout display.

The testing cycle begins when the subject blows moderately hard into the sample inlet tube which is heated to prevent condensation of the breath within. The force of the breath activates a pressure switch which immediately interrupts purging of the sample collection system with room air, and activates an electronic timing sequence. Once the subject has delivered a sufficient breath sample (4 to 7 seconds of moderate blowing), an injector introduces 0.25 ml of the breath sample into a 15 inch long stainless steel column (1/8 inch i/d) heated to 100°C and packed with Porapak Q® as the stationary phase. The breath sample is then carried down the column by a carrier gas composed of 40% hydrogen and 60% nitrogen. The alcohol, separated from the breath as it passes down the column, is quantified by a FID as it elutes from the distal end of the column. The detector signal is amplified and is simultaneously transmitted to the strip chart recorder and the digital readout display. Purging of the instrument begins automatically when the 90 second analytical cycle is complete. Room air is supplied by an internal pump to both support the FID flame, and also to purge the instrument between breath sampling.

For each day of testing, a calibration curve was constructed based upon the results of five standard alcohol solutions. The five different solutions were made up to provide readings of 20, 40, 60, 80 and 100 mg/210 L of breath. For each of the standard solutions, five analyses were conducted, the actual results then plotted on graph paper against the expected result. The procedure followed was to begin with the 100 mg/210 L standard, analyze it once, immediately followed by analysis of the subject's breath sample. This was followed by analysis of the standard again, and then the subject's breath. Such cycling continued until five analyses of the standard had been completed. Analyses then continued with only the subject's breath being analyzed until it had decreased to 80 mg/210 L. Cycling of the 80 mg/ 210 L standard and the subject's breath commenced until five analyses of the standard had been completed. This procedure continued until the subject's breath had

declined to about 20 mg/210 L, and five analyses of all standards had been complete.

A calibration curve was constructed from the analytical results of the standard alcohol solutions. The formula for the best fitting line to the paired data points was determined by linear regression analysis. The resulting formula was then used to correct each of the subject's breath results.

Intoximeter 3000 (IR3000)

The IR3000 employs the principles of non-dispersive infrared molecular absorption to quantify the alcohol concentration in breath samples. The analysis is totally automated and is controlled by an inboard computer. The analytical cycle is initiated by typing in the name of the subject on the attached key board. The instrument then automatically begins a cycle of analyzing room air, analyzing an internal standard, and analyzing room air again before requesting the subject to provide a breath sample. Should the result of the internal standard not be within tolerances, the instrument will automatically abort the analysis.

The subject needs to blow moderately hard for about six seconds through a heated, retractable sample inlet tube. If residual mouth alcohol is detected, or an insufficient sample collected, the instrument will automatically abort. Once the sample has been collected, the instrument automatically analyzes it and prints out the result on heat sensitive paper. The sampling and analysis cycle lasts about two minutes.

The sample cell is divided into two chambers. The right chamber is the sample chamber, with the left being the reference chamber. During the analysis, a single source beam of infrared light with an absorption band of 3.39 microns (modulated at 180 Hz and 300 Hz) is split and is sent down both chambers. When there is no absorbing gas in the right sample chamber, the energy of the sample beam is ratioed against the energy passing through the reference chamber. The ratio so established sets the base line zero set point. The introduction of alcohol into the sample chamber will result in some of the infrared energy being absorbed. The amount of energy attenuated is proportional to the alcohol concentration in the sample.

Monthly calibration checks of the IR3000 have shown it to be a very stable instrument. The following figure shows a typical calibration check for the IR3000.

Calibration Check for IR3000

	ALCOHOL STANDARD (mg/210 L)			
ANALYSIS NO.	20	40	70	100
1	20	41	71	101
2	18	39	70	102
3	18	40	70	102
4	19	39	70	103
5	19	40	71	103
MEAN	18.8	39.8	70.4	103
± SD	0.8	0.8	0.6	1.3
Formula for Graph $y = 1.04x - 2$ ($P < .001$, $r^2 = .9997$)				

Prior to each day of testing, and immediately following the testing session, a "self test" is conducted on the IR3000. This provides a check of the printer, the amount of usable random access memory available, a program check (ROM check), and a check of the input and output signals passing between the interface board and the micro processor. As well, a quantitative test is made on the analog signals coming into the computer from the IR module, and a reference channel check is conducted. A report is printed and the results of the test are checked to make sure the instrument is operating within its tolerances.

Accuracy and Precision of Breath Testing Instruments

In the present study, the range of breath alcohol concentrations was limited to between 20 and 130 mg/210 L of breath. Within this range the accuracy of both the GCI and the IR3000 was within ± 5%. The precision of both instruments was within ± 3 mg/210 L.

In terms of calibration, the GCI required calibration prior to each testing session. The IR3000, however, has proven to be extremely stable and has not required recalibration in over four years of operation.