

PROFILES IN FORENSIC TOXICOLOGY

Professor Erik Widmark (1889-1945)

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Most members of TIAFT have probably heard the name of Erik Widmark, especially those with an interest in forensic pharmacology and toxicology of ethanol. He was a Swedish pioneer in medicolegal aspects of alcohol during the first half of the 20th century and was appointed Professor of Medical and Physiological Chemistry at the University of Lund in Southern Sweden at the unusually young age of 31 y. Widmark remained scientifically productive until his untimely death in 1945 aged 55 y. In 1922 he published a paper describing a quantitative method for the determination of ethanol in 80-100 mg of fingertip blood. This involved micro-diffusion of volatiles followed by oxidation with chromic acid and iodometric titration to detect the endpoint. This method was sufficiently reliable to allow the Swedish government to introduce a statutory blood-alcohol concentration (BAC) limit for driving in 1941. Furthermore, Widmark was a pioneer in the development of pharmacokinetics (PK) and he introduced concepts such as zero-order and first-order kinetics, elimination half-life and volume of distribution for drugs like acetone, ethanol and methanol. The results of Widmark's alcohol research are still relevant today when forensic toxicologists testify in court and interpret a person's BAC in relation to the amount of alcohol consumed and the degree of impairment of body functions.

Introduction

The name of Erik Widmark is tightly linked with quantitative studies of absorption, distribution, metabolism and excretion (ADME) of ethanol and the introduction of statutory BAC limits for driving. Alcohol is the psychoactive substance most often encountered in forensic casework when toxicologists investigate unnatural deaths (post-mortem cases), crimes involving drug-facilitated sexual assaults and impaired driving. Over-consumption of alcohol and drunkenness are major public health problems resulting in premature death and disability. Alcohol kills as a result of acute intoxication (poisoning), blunt trauma from accidental falls and violence when under the influence of alcohol, alcohol-related road-traffic crashes and accidents at home and in the workplace, as well as cumulative damage to body organs and tissue (e.g. cirrhosis and pancreatitis) after years of heavy drinking.

Analytical methods for the determination of ethanol in body fluids appeared during the first decades of the 20th

century, such as Widmark's micro-diffusion method, which was published in 1922 [1]. Use of this method required that volatile substances, such as ethanol, were first removed from the blood by diffusion in especially constructed glass flasks followed by oxidation with chromic acid and volumetric analysis with iodometric titration to detect an endpoint.

Research published by Widmark during the 1920-1930s laid the foundation for our current understanding of ethanol pharmacokinetics. Use of the "Widmark equation" made it possible to calculate the amount of ethanol absorbed and distributed in all body fluids and tissues from the concentration determined in a sample of a person's blood. Most of Widmark's early publications were written in German, including his micro-diffusion method of blood-alcohol analysis "*Eine Mikromethode zur Bestimmung von Äthylalkohol im Blut*" [1]. Likewise, his famous monograph about medicolegal aspects of alcohol "*Die theoretischen Grundlagen und die praktische Verwendbarkeit der gerichtlich-medizinischen Alkoholbestimmung*" was published by



FIGURE 1 (ABOVE LEFT). ERIK MP WIDMARK (1889-1945) PROFESSOR OF MEDICAL AND PHYSIOLOGICAL CHEMISTRY AT THE UNIVERSITY OF LUND, SWEDEN FROM 1920-1945.

FIGURE 2 (ABOVE RIGHT). THE HOUSE IN CENTRAL LUND (BYTAREGATAN 17) WHERE ERIK WIDMARK LIVED AND WHICH WAS LATER OCCUPIED BY HIS SON DR. PER-HENRIK WIDMARK. THE BRIEFCASE ON THE FRONT STEP BELONGS TO THE AUTHOR OF THIS ARTICLE.

Urban & Schwarzenberg Verlag (Berlin and Vienna) in 1932 [2]. Before WW2 it was the standard practice for scientists from the Scandinavian countries to write their dissertations in German and to submit articles for publication in German language journals.

Long before the word 'pharmacokinetics' was coined in 1953 [3], Erik Widmark had published seminal articles about the disposition and fate in the body of drugs such as acetone and ethanol, which he referred to as the neutral or indifferent narcotics [4]. He plotted the concentration-time (C-T) profiles of ethanol and acetone and evaluated these curves in a quantitative way demonstrating that ethanol was metabolized according to zero-order kinetics, whereas acetone followed a first-order reaction process [5] [6].

Neophyte toxicologists encounter the name of Widmark early in their training because excessive drinking and drunkenness are so common in forensic investigations of living and deceased persons. Expert witnesses are asked to interpret a person's BAC in relation to the amount of alcohol consumed and the degree of impairment of the individual. Another question that often arises in some jurisdictions is to back-extrapolate a person's BAC from the time of sampling blood to an earlier time, such as the time of driving or when an alleged sexual assault occurred. However, making such a back-calculation is a controversial practice in criminal cases because there are so many variable factors that need consideration to ensure reliable and realistic results.

Forensic practitioners need to be aware of historical developments in the science of toxicology and this includes the methods for analysis of ethanol in body fluids and how the results should be interpreted in relation to the amount of alcohol consumed and degree of impairment of body functions. The present Forensic Toxicology Profile reviews the life and accomplishments of Professor Erik MP Widmark of Sweden.

Background, education and training

Erik Matteo Prochet Widmark (figure 1) was born in Helsingborg, a small town on the south coast of Sweden, 13 June 1889. He was the youngest of three brothers and his ancestors originally came from the northern part of the country (Umeå).

They were known for diligence, hard-work and versatility. One of Widmark's uncles became Professor of Ophthalmology at the University of Stockholm and he managed to publish original work on the biological effects of ultraviolet radiation.

As a youth, Widmark was especially keen on biology and his hobbies included the collection of butterflies and beetles; he was much attracted to nature and the outdoor life. After completion of high school (gymnasium), he enrolled in 1907 at the University of Lund to study for a degree in zoology. However, following encouragement by his teachers he switched to the medical faculty where better opportunities existed to engage in original scientific research. His medical studies were successful and Widmark obtained a license to practice medicine in 1916, although by this time he was already committed to a career in research and had started studies towards his doctoral degree.

Widmark's first publication on alcohol dates from 1914 and the Swedish title was "*Om alkoholens öfvergång i urinen samt om en enkel, klinisk användbar metod för diagnosticering af alkoholförekomst i kroppen*" [7]. Translated into English this becomes "*On the passage of alcohol into the urine and a simple clinical method to diagnose the presence of alcohol in the body.*" This article marked the first of many future publications about the physiology and pharmacology of alcohol and diagnosis of intoxication based on measuring the concentration of ethanol determined in body fluids.

Doctoral thesis on acetone and ketoacidosis

Erik Widmark spent his entire career as a student, teacher and professor at the University of Lund (founded in 1666); at the time one of only three medical schools in Sweden. The Widmark family lived in central Lund and the house was located on Bytaregatan 17 as shown in figure 2. After Widmark's death the house was occupied by his son, Dr. Per-Henrik Widmark, who was a local medical practitioner.

During his medical studies, Widmark became an unpaid assistant (amanuens) in the Department of Physiology, which was headed by Professor Torsten Thunberg (1873-1952). Thunberg had managed to attract to his department an enthusiastic group of young collaborators, one of whom was Erik Widmark. During this period it was customary that the

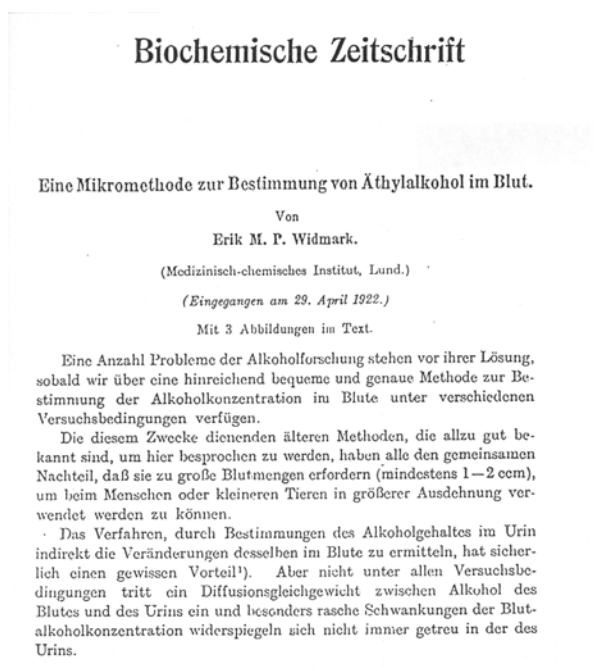
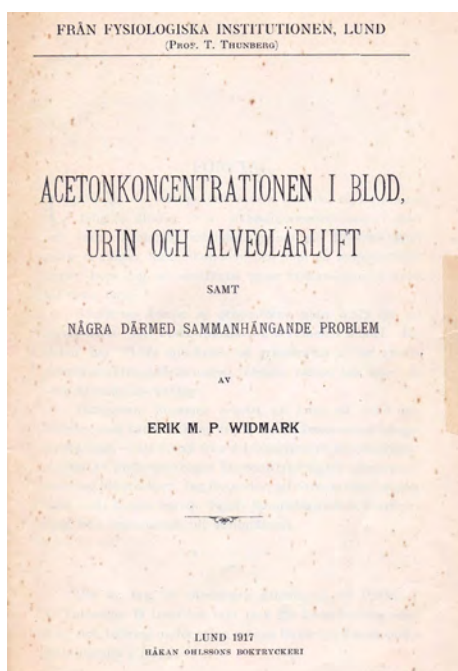


FIGURE 3. TITLE PAGE (LEFT) FROM WIDMARK'S THESIS FOR HIS MD DEGREE ENTITLED "THE CONCENTRATIONS OF ACETONE IN BLOOD, URINE AND ALVEOLAR AIR", WHICH WAS PUBLISHED IN 1917 AND FIRST PAGE FROM A 1922 ARTICLE DESCRIBING A MICRO-DIFFUSION METHOD FOR THE DETERMINATION OF ETHYL ALCOHOL IN BLOOD (RIGHT).

student suggested a suitable research topic to investigate for a PhD or MD degree after consultation with senior scientists within the medical faculty.

It appears that Widmark was influenced by work done at the department of pharmacology, which was headed by Professor Charles Ernst Overton (1865-1933), who had published important articles on the interaction between organic solvents and cell membranes [8]. Overton was British by birth but received his training in research in Zurich and Würzburg, before being "called to the chair" of pharmacology in Lund. He is probably best remembered for the Meyer-Overton theory describing the interaction of narcotic drugs with cell membrane lipids and the theory of anesthesia [9]. This research was described in a classic work entitled "*Studien über die Narkose*" (Studies of narcosis), which was published in 1901 before his appointment at the University of Lund in 1907 [10].

In the preface to his thesis, Widmark mentions that he became interested in acetone from his earlier work with ethanol intoxication, and he turned his attention to acetone because this was more lipid soluble than ethanol. Acetone is produced endogenously as one of the ketone bodies that are generated during the metabolism of fatty acids. When the supply of dietary carbohydrate is limited or the body is unable to utilize glucose as a metabolic fuel, this causes lipolysis and the synthesis of ketone bodies in excess. The fruity smell of acetone on a person's breath is a prominent sign of starvation and diabetes. Widmark considered that the analysis of acetone might serve as a useful biomarker to judge the severity of diabetic ketoacidosis.

The hallmark of Type 1 diabetes is elevated blood sugar level and before the discovery of insulin in 1922 by Banting and Best in Toronto, this was a certain death sentence [11, 12]. The extraction, purification and clinical use of the pancreatic hormone insulin ranks as one of the greatest benefits to mankind [13]. Type 1 diabetes or juvenile diabetes is usually diagnosed in childhood and there is a total deficiency in the production of insulin by the pancreatic β -cells, which seemingly

become destroyed by an auto-immune reaction [14]. Without insulin, the body cannot regulate the blood sugar after meals and hyperglycemia develops and the associated diabetic ketoacidosis becomes a medical emergency.

To determine acetone in body fluids, Widmark developed a micro-diffusion method taking advantage of the chemical reaction between a carbonyl group and elemental iodine in alkaline solution which leads to the formation of iodoform (CHI_3). Acetone was first separated from the biological matrix by distillation and to this was added a mixture of iodide and potassium hydroxide, which resulted in production of iodoform. When the chemical reaction was complete, the solution was acidified which liberated excess iodine and was then determined by titrimetric analysis and sodium thiosulfate.

The use of breath samples for analysis of acetone was investigated by Widmark, because it provided a non-invasive method to monitor the severity of ketoacidosis in diabetic patients. The fate of acetone in the body was investigated by allowing healthy volunteers to drink small amounts of acetone diluted with water and then taking repetitive samples of blood, breath and urine for analysis. Widmark determined the main pharmacokinetic parameters of acetone including elimination rate constant and terminal half-life, which was of the order 18-22 h. The exhalation of acetone in breath was tested in hospital patients, thus predating the later much more common application of breath-ethanol analysis for testing drunken drivers

Widmark's thesis (figure 3) was written in Swedish entitled "*Acetonkoncentrationen i blod, urin och alveolärluft samt några därmed sammanhängande problem*" [15] or "*The concentrations of acetone in blood, urine and alveolar air and some related problems.*" The work was accepted by the medical faculty in May 1917, so this year (2017) marks the centenary of Widmark's doctoral dissertation.

Early academic conflicts

The path towards a successful academic career in Sweden



FIGURE 4. IN 1920 ERIK WIDMARK WAS APPOINTED PROFESSOR OF MEDICAL AND PHYSIOLOGICAL CHEMISTRY AT THE UNIVERSITY OF LUND AT THE UNUSUALLY YOUNG AGE OF 31 Y. HE IS SHOWN HERE AT WORK AT HIS LABORATORY BENCH (LEFT) AND SITTING AT HIS WRITING DESK WITH LABORATORY COAT HANGING ON THE WALL BEHIND (RIGHT).

depended to a large extent on obtaining high marks for work contained in the doctoral thesis. This includes the written part of the thesis, the published articles based on the thesis and the public defense of the work before the medical faculty and an external examiner (opponent). Unfortunately for Widmark during the defense of his thesis, strong opposition arose from a senior member of the medical faculty in Lund, namely Professor Ivar Christian Bang (1869-1918). Bang is considered a pioneer in the field of clinical chemistry and biochemistry, so his negative attitude and hostility towards Widmark was a serious problem. Bang found faults with the analytical method used by Widmark to determine acetone in micro-samples of blood and if this method was unreliable then the entire thesis including the clinical work with diabetics was jeopardized [16, 17].

There was certainly no love lost between Professor Bang and Dr. Widmark as evidenced by a number of acrimonious written exchanges after the thesis was initially rejected by the medical faculty. To settle the argument, the University of Lund appointed two independent experts, one from Norway and the other from Denmark, to review the thesis and to consider Professor Bang's critique. Both outside experts found that Professor Bang's critique was unfounded and wrote reports supporting Widmark who eventually obtained high marks for his thesis. The following year he was appointed docent (assistant professor), which marked the starting point for a career in academic medicine as an independent researcher. What actually motivated the strong opposition from Professor Bang will never be known, but one possibility is inter-departmental rivalry. Widmark did the research for his thesis at the Department of Physiology, whereas Professor Bang headed the Department of Medical and Physiological Chemistry. Perhaps Bang considered that Widmark was trespassing on his own special area of expertise, namely micro-analysis of endogenous substances, such as glucose, protein, and lipids in blood and plasma [16].

The results from Widmark's thesis were summarized in three original articles published in *Biochemical Journal* [18-20]. The choice of a British journal was unusual at the time because most Swedish scientists sent their work for publication to German language journals. The decision to write the papers in English might have been a way to avoid that Professor Bang was invited to serve as a peer reviewer, because he was much better known in German scientific circles than he was in the UK.

Full professor aged 31 years

As fate would have it, Widmark's nemesis Professor Bang died suddenly from a coronary embolism, while working in his laboratory at the age of just 49 y. The chair of Medical and Physiological Chemistry therefore became vacant and was opened for applications. Widmark along with three other candidates applied for the position although the medical faculty in Lund had decided to invite a German scientist, namely Leonor Michaelis (1875-1949) to become successor to Professor Bang.

The name of Leonor Michaelis is probably known to some TIAFT members from the Michaelis-Menten equation, which deals with the kinetics of enzyme-substrate reactions as described in a classic article from 1913 [21]. This work was done in collaboration with Maud Menten (1879-1960), who came from Toronto to work at Michaelis' laboratory in Berlin [22]. Dr. Menten eventually returned to live in North America and became one of the first female scientists to be appointed full professor at the University of Pittsburgh.

The decision "to call" Michaelis to occupy the professorship after Bang's sudden death caused considerable debate and unrest among young academics at Swedish universities at the time. This policy was considered unacceptable in a small country like Sweden where few opportunities existed to reach high academic ranks and promotion to professorships. The Swedish Ministry of Education became involved in the debate, which was also closely followed by the national newspapers. One requirement necessary before a foreign scientist was "called to" a professorship in preference to a qualified Swede was that the person's research was of an exceptionally high standard, so-called "utmärkt förtjänst." To settle this question, the publications of Michaelis were sent for review to experts from other Nordic countries, such as Denmark and Norway. They did not consider that Michaelis' work at the time had reached such a high standard of "utmärkt förtjänst." Accordingly, the Swedish government overruled the decision by the University of Lund, which meant that at the unusually young age of 31 y Dr. Erik Widmark became Professor and Chairman of the Department of Medical and Physiological Chemistry in 1920; a position he held until his death in 1945 (figure 4).

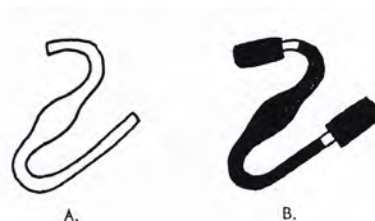
Leonor Michaelis eventually left Germany in the 1920s to take-up an appointment as professor of biochemistry in Japan (Nagoya) and he later moved to live and work in the USA where



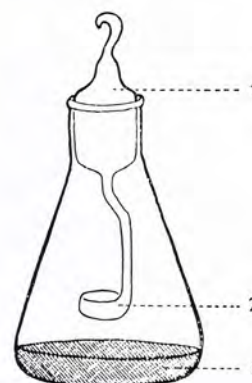
SMALL METAL CONTAINERS USED TO SEND SIX BLOOD CAPILLARIES TO A CENTRAL LABORATORY FOR ANALYSIS.



SAMPLING OF FINGERTIP CAPILLARY BLOOD FOR ANALYSIS OF ALCOHOL BY WIDMARK'S METHOD.



A. B.



EMPTY (A) AND FILLED (B) CAPILLARY TUBES (ABOVE) AND THE WIDMARK MICRO-DIFFUSION FLASK (BELOW).

FIGURE 5. GLASS S-SHAPED CAPILLARY TUBES USED TO SAMPLE FINGERTIP BLOOD FOR DETERMINATION OF ETHANOL. THESE "BLODPROV FÖR ALKOHOLANALYSIS" (TOP LEFT) WERE USED IN SWEDEN UNTIL 1980. EACH TUBE HELD 80-120 MG OF BLOOD AND WERE TAKEN BY PRICKING A FINGERTIP (BOTTOM LEFT) AND SENT TO A CENTRAL LABORATORY FOR ANALYSIS. TO THE RIGHT ARE SHOWN EMPTY (A) AND FILLED (B) BLOOD CAPILLARY AS WELL AS A MICRO-DIFFUSION FLASK. THE NUMBERING ON THE FLASK SHOWS GROUND GLASS JOINT WITH HOOK (1), GLASS CUP THAT CONTAINED A WEIGHED ALIQUOT OF BLOOD (2) AND THE DICHRIMATE-SULFURIC ACID OXIDIZING AGENT (3).

he died in 1949. His overall body of work in biochemistry did eventually reach a high scientific standard considering that he was elected to membership in the US National Academy of Sciences and was also nominated nine times for a Nobel Prize in physiology or medicine between 1923 and 1949 [23].

Determination of ethanol in blood

Nobody knows for sure when the first humans (*Homo sapiens*) encountered alcohol, but this was probably by accident in their never ending search for food, energy and survival. Ethanol is produced when the carbohydrates contained in natural products, such as grapes, berries, dates and honey, undergo fermentation. The first hunter-gatherers must surely have occasionally eaten fruits infected by molds, yeasts or other micro-organisms so that sugars were fermented. This makes ethanol man's oldest psychoactive drug and good evidence exists that in parts of China alcoholic beverages were produced by fermentation 7000 BC, as demonstrated by the discovery of chemical residues in archeological artifacts [24].

The oxidation of ethanol to acetaldehyde was discovered in 1835 by the great German chemist Justus von Liebig (1803-1873), as reported in an article entitled "*Über die Produkte der Oxidation des alkohols.*" He used an oxidizing agent consisting of a mixture of a chromate or dichromate salt and strong sulphuric acid thus producing chromic acid in-situ. The same oxidizing agent was used by Dr. Francis Anstie (1833-1874) in 1865 when he demonstrated that only a small fraction of the alcohol consumed was excreted unchanged in breath, sweat and urine [25].

Oxidation of ethanol with chromic acid was later utilized by many scientists including Dr. Maurice Nicloux (1873-1945)

from Strasbourg, who was very active in medicolegal alcohol analysis during the first half of the 20th century [26]. However, the micro-diffusion method developed by Widmark was much more practical for routine purposes and required only 80-100 mg of blood sample allowing serial determinations and kinetic studies (figure 5).

The blood sample was taken by pricking a fingertip or earlobe (capillary blood) with the help of specially prepared S-shaped glass tubes coated on the inside with a mixture of fluoride and oxalate as anticoagulant and preservative. The aliquots of fingertip blood were weighed on a torsion balance so the final BAC was reported in concentration units of mass/mass (mg/g or g/kg). The known amount of blood was transferred into a small glass cup fitted to the stopper of the micro-diffusion flask. A known volume of the dichromate-sulfuric acid mixture was added to the flask, which was then heated to 50°C for a few hours. This caused any volatiles in the blood to react with the chromic acid reagent, which was in excess and the amount of oxidizing agent remaining was determined by iodometric titration with standard thiosulphate solution. During the 1930s scientists from several laboratories in Germany, Switzerland and the Nordic countries visited Widmark's laboratory at the University of Lund to learn about this analytical method, which was later widely acclaimed as a quantitative technique and used in forensic applications.

To commemorate the 50th anniversary of TIAFT, Alan Verstraete edited a book about the history of our organization. One of the chapters in the book contained an historical survey of GC methods used for blood-alcohol analysis, especially GC-FID and HS-GC-FID, which are still in use today [27]. Over the past 100 years, the methods used to determine blood-ethanol in clinical and forensic medicine can be subdivided into four

basic principles or strategies, namely:

(i) Chemical oxidation with a mixture of potassium dichromate and sulfuric acid (chromic acid), such as the micro-diffusion method published by Widmark in 1922 and described above [1].

(ii) Enzymatic oxidation with liver or yeast alcohol dehydrogenase as described by Bonnichsen and Theorell in 1951 [28].

(iii) Gas chromatography with flame ionization detector after dilution of blood with internal standard, either by direct injection of blood sample or headspace sampling, as reported in the 50th anniversary book of TIAFT in 2013 [27].

(iv) Gas chromatography with a mass selective detector focusing on molecular ion (m/x 46) base peak (m/z 31) and qualifier ion m/z 45 [29].

Post-mortem alcohol analysis

In collaboration with the Department of Forensic Medicine in Lund, which was headed by Professor Einar Sjövall (1879-1964), Widmark investigated problems associated with determination of ethanol in post-mortem specimens [30]. Experiments were designed to investigate the stability of ethanol in the body after death with rabbits as an animal model. After administration of a known dose of ethanol, blood was drawn for analysis before the animals were killed and the bodies were allowed to stand at room temperature for various periods of time and blood samples again taken for analysis. The results showed that after death the BAC initially decreased, but after a few days began to increase as the post-mortem interval increased, which suggests a neo-formation of ethanol. Experiments were also done with human cadavers when death was attributed to drowning, road-traffic fatality, or acute alcohol poisoning. The investigators recommended that peripheral blood was preferred to cardiac blood, owing to the risk of diffusion of alcohol from the stomach contents after death. Additionally, Widmark considered that

urine should be analyzed, because the UAC/BAC ratio could furnish useful information about the time of last ingestion of alcohol before death [31].

The chromic acid oxidation method of analysis was not specific for identification of ethanol, which meant that other volatile substances that might be generated during decomposition and putrefaction processes led to falsely elevated BAC results. This often required preliminary chemical tests to remove aldehydes and ketones from the specimen prior to oxidation and quantitative analysis of ethanol in blood. With today's HS-GC-FID methods lack of analytical specificity is not a major problem, but care is still needed when the results of post-mortem alcohol analysis are interpreted. With an appropriate substrate, such as blood glucose, ethanol can be produced in the vascular system after death by fermentation. In some special cases, a normal blood-glucose concentration of 0.1 g% (100 mg%) can result in blood ethanol of 0.05 g% (50 mg%) in a putrefied corpse [32].

Alcohol and traffic safety

The role played by over-consumption of alcohol as a contributing factor to road-traffic crashes was well recognized already in 1904 not long after automobiles (motor wagons) first became available. In 1914 Widmark suggested that ethanol should be analyzed in urine from the driver to support any clinical signs and symptoms of intoxication. He argued that finding a high concentration of ethanol in urine could help to distinguish drunkenness from a medical condition, such as skull trauma from involvement in a crash, or hypoglycemia, conditions that require immediate medical treatment.

Widmark's micro-diffusion method of blood-alcohol analysis gained widespread acceptance in law enforcement in Nordic countries and in Austria, Germany and Poland. This led to the enactment of a concentration per se law in Norway in 1936 (0.50 ‰ or 0.05 g%) and in Sweden in 1941 (0.80 ‰ or 0.08 g%). Evidence for prosecution of traffic offenders also included examination of suspects by a physician, who administered simple tests to document signs and symptoms of drunkenness.

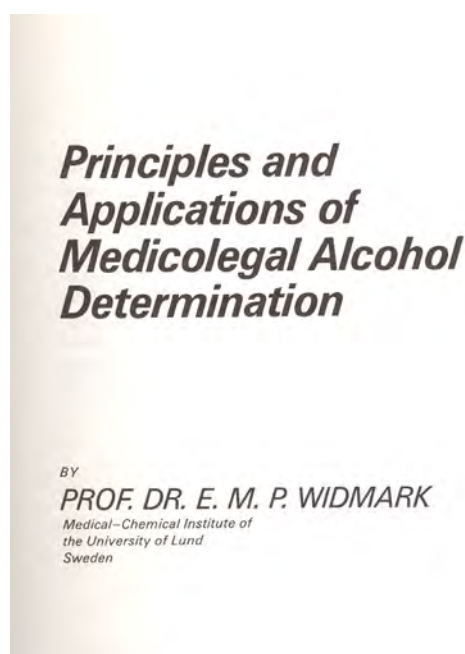
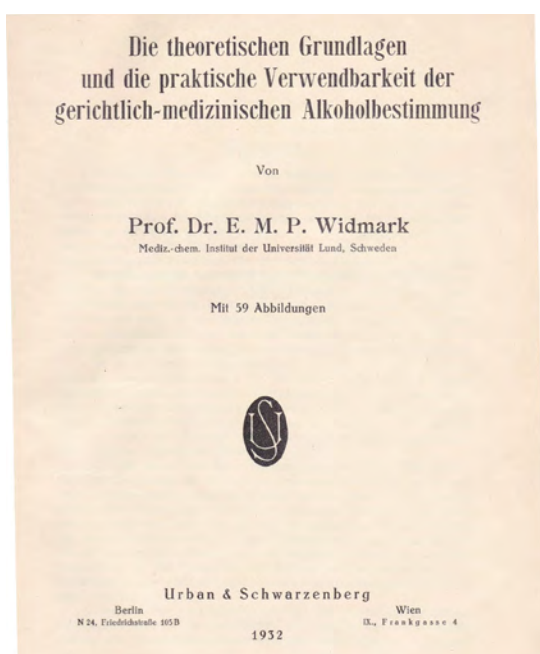


FIGURE 6. FRONT PAGES FROM WIDMARK'S FAMOUS GERMAN MONOGRAPH ON MEDICOLEGAL ASPECTS OF ALCOHOL FIRST PUBLISHED IN 1932 (LEFT) AND AN ENGLISH TRANSLATION OF THE TEXT MADE ~50 Y LATER (1981).

Questions were asked about the person's general state of health, recent use of alcohol and/or other drugs as well as performing certain cognitive and psychomotor tests. However, experience from evaluating results from thousands of such clinical tests showed that they were poorly correlated with a person's BAC. Much seemed to depend on skill, training and experience of the examining physician, the time after drinking when the examination was made (Mellanby effect) and the degree of habituation to alcohol in the arrested driver.

After a concentration per se law was enacted in Sweden in 1941, the courts started to give much more weight to evidence about the driver's BAC and not the clinical examination. In addition to reporting BAC during a trial, it was also customary to calculate the quantity of alcohol absorbed and distributed in all body fluids and tissues and total amount of alcohol the driver had consumed at the time of driving. This was done as described by Widmark and the driver's BAC was converted to the volume of liquor (40% alcohol) necessary to achieve such a result along with a 95% confidence interval.

The per se BAC limit in Sweden was set at 0.80 ‰ (80 mg/100 mL or 0.08 g%) in 1941, although was subsequently lowered to 0.50 ‰ in 1956 and to 0.20 ‰ in 1990, where it stands today. After this road-traffic act was approved, responsibility for blood-alcohol analysis in Sweden was handed over to the National Laboratory of Forensic Chemistry in Stockholm, which at the time was headed by Professor Erik Wolff (1891-1971).

Information about Widmark's forensic alcohol research at the University of Lund spread to other nations as evidenced by a letter he received in 1931 from the US Department of Justice, Bureau of Prohibition. The chief of the research division E.P. Sanford wrote asking various questions about the use of chemical tests for intoxication and experiences from Sweden. Part of the letter read:

"Such tests in our various states vary from smelling the offenders breath to making him walk a chalk line, but no scientific test apparently is applied. We understand that in Sweden or Germany, or in both countries, a blood test is made at the time an offender is arrested that determines quite accurately the amount of alcohol in the blood..... We are particularly anxious to know what the alcoholic content of the blood must be before a person can be

described as being under the influence of alcohol. Some median line must have been established on one side of which an offender is not under the influence of liquor and on the other side of which he may be said to be intoxicated. Just what that line is we should like to know."

The US Department of Justice was worried what might happen when prohibition was repealed, as happened in 1933, making production, distribution and the sale of alcoholic beverages legal, with consideration of the rapid rise in motor transportation on roads and highways throughout the country [33]. Widmark replied to the letter and sent along a number of reprints of articles, including a copy of his German monograph on medicolegal blood alcohol determination.

Widmark's magnum opus

By far the most important of Widmark's publications is a 140 page monograph (figure 6) entitled *"Die theoretischen Grundlagen und die praktische Verwendbarkeit der gerichtlich-medizinischen Alkoholbestimmung"* which was first published in 1932 [2]. This contained a compilation of research results from his earlier studies on acetone and ethanol of medicolegal interest to traffic safety and post-mortem toxicology. This monograph was translated into English and re-published in 1981 entitled *"Principles and applications of medicolegal alcohol determination"* [34]. The roughly 50 year gap between publication of the German and the English versions testify to the longevity of the information and its usefulness to forensic toxicology practitioners today.

Less well known is a 58 page article written by Widmark in French and published in 1930 entitled *"Les Lois Cardinales de la Distribution et du Metabolism de L'alcool Ethylique dans L'organisme Humain"* [35]. In English the title is *"The fundamental laws governing the distribution of alcohol in the human body"* and reported results of numerous controlled human alcohol dosing studies to establish BAC profiles and the pharmacokinetic parameters of ethanol. This French article was obviously a forerunner to the more comprehensive 1932 German publication. It is somewhat of a mystery why Widmark bothered to write the article in the French language, although this might have been his way of bringing it to the attention of Professor

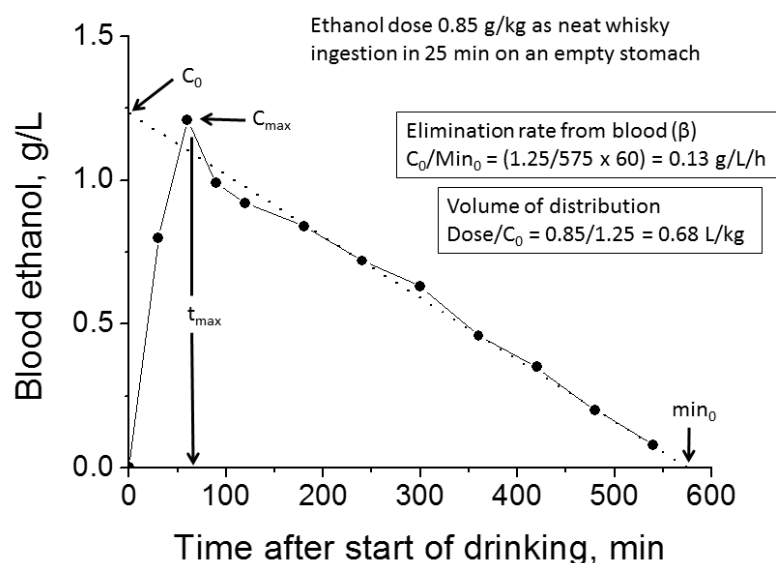


FIGURE 7. BLOOD-ALCOHOL CURVE AND DERIVATION OF THE WIDMARK PHARMACOKINETIC PARAMETERS BETA (ELIMINATION RATE FROM BLOOD) AND RHO (VOLUME OF DISTRIBUTION).

Maurice Nicloux (1873-1945), who worked at the University of Strasbourg. During the first half of the 20th century Nicloux was a highly prolific French scientist in the area of medicolegal alcohol studies and thereby a scientific rival to Widmark.

Human pharmacokinetics

The word pharmacokinetics was coined in 1953 by Friedrich H. Dost (1910-1985) and was mentioned in his book entitled "Der Blutspiegel - Kinetik der Konzentrationsabläufe in der Kreislaufflüssigkeit" [3]. However, the principles of PK applied to drugs such as acetone and ethanol were in detail established by Widmark at the University of Lund in the 1920s.

This is exemplified by a solo authored 28 page article published in *Acta Med Scand* in 1919 where he plotted the concentration-time profiles of acetone after healthy volunteers drank acetone solvent diluted with water. Capillary blood was taken at regular intervals for about 24 h post-dosing and rate of clearance of acetone from blood was determined [36]. A paragraph taken from this article is reproduced below:

"If a certain quantity of acetone is introduced into the body and the acetone concentration in the blood is afterwards tested at varying intervals of time, different values in the acetone concentration will be obtained according to the time that has elapsed from the moment of administration. The curve that can be drawn through the values for these estimates (the concentration curve) shows a rising and a falling part. If the estimates are extended over a period of about a day, the falling part of the concentration curve takes the characteristic form of a logarithmic curve. If in the curve the direct values for the concentrations are exchanged for their logarithms, the observations arrange themselves in a straight line. The fall in concentration in the blood is therefore at every moment proportional to the existing concentration."

This description of what happened to acetone in the body makes it evident that Widmark was a pioneer in pharmacokinetics and that he understood the difference between first-order and zero-order kinetics and the need to make a logarithm transformation to determine a terminal elimination half-life of 18-22 h.

Ethanol pharmacokinetics

Two important pharmacokinetic parameters of ethanol are denoted β and ρ referring to the rate of elimination from blood and the volume of distribution, respectively. These parameters are now a part of the ABC of ethanol pharmacology and toxicology and were derived by Widmark in experiments with 20 healthy men and 10 healthy women. This was followed by an investigation of the effect of drinking alcohol on an empty stomach compared with after a meal, which led him to establish a decreased bioavailability in the fed state, which he ascribed to a "loss of ethanol." He later tested the effects of specific nutrients on ADME of ethanol, such as amino acids, fats and glycerol [37]. Evaluation of the C-T profiles of ethanol demonstrated gender differences in the distribution volume and lower ρ factor in females on average. Quantitative evaluation of C-T profiles of ethanol is shown in figure 7.

Based on these studies, Widmark established the famous Widmark equation (i), which allowed him to calculate the amount of ethanol absorbed and distributed in all body fluids and tissues from the concentration determined in a sample of blood.

$$A(g) = BAC(g/L) \times V_d(L/kg) \times \text{body weight}(kg) \dots\dots\dots(i)$$

In the above equation, A is the amount of ethanol in grams absorbed and distributed in all body fluids and tissues at time of sampling blood. The BAC is the person's blood-alcohol concentration in g/L (not mg/100 mL), and kg is the person's body weight. The ρ factor shown here as V_d is the distribution volume of ethanol in units of liters per kilogram (L/kg). By rearrangement of equation (i) one sees that V_d (L/kg) equals the ratio between alcohol in the whole body (dose = A/kg) and the measured BAC in g/L.

$$V_d(L/kg) = [A(g)/\text{body weight}(kg)]/BAC(g/L) \dots\dots\dots(ii)$$

The same equation can be used to calculate the theoretical maximum BAC obtained if the entire dose is absorbed and distributed in the blood and other body fluids instantaneously without any metabolism taking place by re-arranging equation (i) to give equation (iii):

$$BAC = A/(kg \times V_d) \dots\dots\dots(iii)$$



2dra editionen

**Inbjudan
till
WIDMARK-JUBILEET
11-13 juni 1989
i Lund
med symposiet
ALKOHOL och TRAFIK
i Aulan, Lunds Lasarett**



FIGURE 8. INVITATION (RIGHT) TO PARTICIPATE IN A SYMPOSIUM ON ALCOHOL AND TRAFFIC HELD AT THE UNIVERSITY OF LUND JUNE 1989 TO COMMEMORATE THE 100TH ANNIVERSARY OF WIDMARK'S BIRTH. THE PICTURE OF WIDMARK SHOWN TO THE LEFT WAS TAKEN IN 1930 AGED 41 Y.



FIGURE 9. PHOTOGRAPH OF PROFESSOR WIDMARK IN A MORE LEISURELY MOOD FISHING AND ENJOYING A CIGAR (ABOVE LEFT). TO THE RIGHT IS SHOWN A CARICATURE OF HIM DRAWN BY THE MEDICAL STUDENTS AT THE UNIVERSITY OF LUND.

In the above equation, A is the amount of alcohol consumed in gram, kg is the person's body weight and V_d is the volume of distribution of ethanol (L/kg). The BAC obtained from equation (iii) corresponds to the value of C_0 shown in figure 7.

In reality, however, some of the alcohol ingested gets metabolized in the liver before the blood sample is taken so equation (iii) needs to be modified to account for this, hence equation (iv), where β is the rate of ethanol elimination from blood per hour and t is the time elapsed from start of drinking until the blood was sampled.

$$\text{BAC} = [A/(kg \times V_d) - (\beta \times t)] \dots \dots \dots \text{(iv)}$$

After drinking alcoholic beverages, the bioavailability of ethanol is rarely 100% because part of the dose undergoes first-pass metabolism by enzymes in the gastric mucosa and/or the liver before blood reaches the systemic circulation. Under some circumstances, such as when ethanol is consumed repeatedly over several hours or together with a large meal, the bioavailability of ethanol might be as low as 60%. This needs consideration when Widmark calculations are made in criminal cases. Unfortunately, there are few controlled drinking studies under real-life situations. If equation (iv) is used to calculate the BAC expected after drinking a given dose of ethanol in situations when first-pass metabolism is appreciable, then the results obtained are higher than those found by experiment.

Derivation of the volume of distribution (V_d) of ethanol is shown in figure 7 and average values of 0.68 for 20 men and 0.55 for 10 women were obtained with an inter-subject variation of about 15-20%. Later studies with more drinking subjects found higher average values of 0.70 L/kg for non-obese males and 0.60 L/kg for non-obese females. The lower values in women is explained by their higher proportion of body fat and less body water/kg in the female gender. Taken together, the distribution volume of ethanol in humans might vary by a factor of two, from a low value of 0.40 L/kg, as might be observed in an obese female, to a high value of 0.80 L/kg for a muscular male subject.

The rate of elimination of ethanol from blood (β) also varies between individuals by a factor of about three ranging from 0.10-0.30 g/L per h (10-30 mg/100 mL per h or 0.01-0.03 g% per h). Low values are more likely in people who are malnourished, or who eat low-protein diets or engage in prolonged fasting or forced starvation. High values are observed in heavy drinkers, such as alcoholics after a drinking binge, owing to induction

of a microsomal oxidative enzyme, denoted CYP2E1. Because there are many heavy drinkers and alcoholics in the population of drinking drivers, one can expect higher rates of ethanol elimination from blood. The means BAC in apprehended drivers in most nations is between 1.5-1.7 g/L (0.15-0.17 g%), which can only be reached by binge drinking. This probably accounts for the higher average rate of elimination of ethanol from blood of 0.19 g/L/h (19 mg/100 mL per h) in these traffic delinquents compared with a mean of 0.15 g/L/h in moderate drinkers [38].

Concluding remarks

Professor Erik Widmark was a veritable pioneer in the field of forensic alcohol research as evidenced by scores of publications from the second, third and fourth decades of the last century. The monograph (figure 6) he published in 1932 was a landmark publication and the information it contains remains highly relevant today when expert witnesses testify in court about alcohol pharmacokinetics. Widmark's studies of acetone PK and the determination of in-vitro (air-blood) and in-vivo (breath-blood) partition ratios laid the foundation for the later development of breath-alcohol analysis in traffic-law enforcement.

Some of Widmark's last publications dealt with medical nutrition and the need to eat a well-balanced diet including appropriate amounts of micro- and macro-nutrients. His last publication about alcohol appeared the year he died in 1945 and described use of an animal model with dogs to study health hazards of chronic heavy drinking [39]. Historians of pharmacokinetics, such as Professor John Wagner (1921-1998) from the University of Michigan, recognized Widmark as a pioneer in this realm of pharmacology [40]. Among specialists in legal medicine, especially in Germany, the name of Widmark is held in high esteem. The scientific journal *Blutalkohol* is devoted to publishing articles about alcohol, drugs and behavior, especially drunken driving and traffic safety, which are areas of research started by Widmark [41].

For those who would like to read more about the life and work of Professor Widmark, there are a number of articles available. A colleague of mine, the late Dr. Rune Andreasson (1920-2013), wrote a biography of Widmark with main focus on his contributions to alcohol and traffic safety. A symposium was held at the University of Lund in 1989 to commemorate the 100th anniversary of Widmark's birth (figure 8). I attended

and presented a paper describing recent advances in methods of forensic alcohol analysis with main focus on the use of alternative specimens.

In 1995 exactly 50 years after Widmark's death Andreasson and Jones wrote a tribute to his life and work that appeared in *Forensic Science International* [42]. Shortly afterward the editor of the *American Journal of Forensic Medicine and Pathology* (Dr. Vincent DiMaio) invited me to write a similar article for publication in his journal, which was published in 1996 [43]. On the occasion of the first joint TIAFT-ICADTS meeting, which was held in Seattle in 2000, I presented a plenary lecture describing how Widmark's work managed to bridge the gap between forensic toxicology and traffic safety research [44].

Erik Widmark was popular with the medical students in Lund and for many years he functioned as the faculty representative at the student's union. He participated in many of the student activities, including celebrations, dinner parties, festivities and other events (figure 9). He also encouraged the students to participate in sports, such as tennis, golf, swimming and fishing, as an important way to relax and as a complement to their academic studies.

The importance of Widmark's scientific research and publications was recognized in 1938 when he was elected to membership of the Royal Swedish Academy of Sciences (Kungliga Vetenskapsakademien). This distinction is equivalent to being a Fellow of the Royal Society (FRS) in UK or the National Academy of Sciences in USA. Widmark's bibliography lists about 100 original articles and reviews from several research domains including acetone and ketoacidosis, ethanol pharmacokinetics, medical nutrition and vitamins, use of animal models in alcoholism studies, the clinical diagnosis of intoxication and the relationship between BAC and impairment, as well as problems with postmortem blood-alcohol analysis.

Professor Widmark remained scientifically productive until his death in 1945, although those close to him could not help noticing that his health was deteriorating. He was overweight, smoked cigars and suffered from hypertension; the death certificate mentions arteriosclerosis as a contributing factor. He passed away on 30 April 1945 a few months before reaching the age of 56 y. However, his scientific legacy is secured thanks to scores of landmark publications about acetone and ethanol and efforts to improve traffic safety by prosecuting drunken drivers. In 1965 the International Council on Alcohol, Drugs and Traffic Safety (ICADTS) created the "Widmark Award" to honor those members of the organization who have made career-long contributions to research and scholarship on the subject of alcohol, drugs and driving.

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TIAFT GRANTS FOR ANALYTICAL METHOD DEVELOPMENT

TIAFT is providing support to young scientists to assist in developing analytical methods in a foreign laboratory, increasing toxicology capacity and international networks. The procedure to apply for the grant is as follows:

A member from a lab must send a request to the President, containing the following information:

- Name of the lab where the method will be developed.
- Method that needs to be developed: target analytes, type of instrument, detection method (mass spectrometric or not), requested detection limits, ...;
- Equipment on which the methods will be developed.
- A list of equipment and reagents that is available in the lab. This includes standards, internal standards, buffers, solvents, analytical instrumentation, pipettes, columns, et cetera. All the equipment should be present in the lab when the application is made. The analytical instruments, like gas or liquid chromatographs, should be fully installed and operational. The request should contain pictures of the instrumentation as it is installed in the lab.

- An estimation of the time needed for the young scientist to develop the method.
- The name(s) and CV of the person(s) who will be present to assist the young scientist during his stay.
- A list of the languages that are spoken by the staff.
- An estimation of the costs for food and lodging for the young scientist, and a description of how this will be arranged.

The president submits the proposal to the Board. If approved, the president asks the President of the Young Scientists Committee to find a young scientist who is ready to go to the lab to develop the method.

When this person has been selected, he/she agrees on the dates with the lab and submits a budget for the transportation to the Treasurer. If the treasurer approves the budget, the ticket can be purchased. After the stay, the member who has submitted the proposal writes a short report for the board and the Bulletin.