# A COMPARATIVE STUDY OF THE RELIABILITY OF SEVERAL CHEMICAL TESTS FOR ETHYL ALCOHOL INTOXICATION IN HUMANS

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# A COMPARATIVE STUDY OF THE RELIABILITY OF SEVERAL CHEMICAL TESTS FOR ETHYL ALCOHOL INTOXICATION IN HUMANS

BY

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## INTRODUCTION

"Accidents constitute the most serious death loss to society, outranking such leading causes of death as heart disease, apoplectic stroke and cancer." (1). The alarming number of traffic accidents involving drivers and pedestrians suspected to be under the influence of ethyl alcohol* has stimulated interest in further study of the reliability and comparability of chemical tests for intoxication.

Ethyl alcohol is known to be a nerve depressant, not a stimulant as it has often been regarded. The amount of alcohol in the circulatory system of a person's body will have a direct bearing on the degree of impairment of the normal sensory and motor nerve functions of that subject. Increasing the blood alcohol concentration will also increase the degree of impairment of normal nerve impulses. The need of chemical tests as a measure of the concentration of ethyl alcohol in the blood stream has long been recognized as an aid in measuring the degree of intoxication. (2,3,4,5,6)

Methods for the analysis of body fluids for alcohol have been described and used in many countries. Some European countries** have

* Ethyl Alcohol mentioned in this paper refers to the intoxicating substance contained in alcoholic beverages, e.g. beer, whiskey, wine and etc. It can be isolated and identified in the free form from either alcoholic beverages or body fluids after consumption of substances containing ethyl alcohol.

**Sweden, Great Britain and Germany have enacted legislation for use of chemical tests for evidence in case of intoxication.

accepted these tests as reliable, whereas only a relative small number of the states in the United States have enacted legislation which permits the use of chemical tests for intoxication as evidence in court cases.

"Chemical testing for alcohol concentration is now almost a century old, for it dates from Civil War times. But, as has been true with respect to the admissibility of all scientific implements of enforcement, one great hurdle had to be overcome before real evidence attained as a result of a scientific measure would be admitted in court.

The scientific principle had to cross the line from the twilight zone of experimentation to a demonstrable stage (7). 'Just when a scientific principle or discovery crosses the line between the experimental and demonstrable stages is difficult to define. Somewhere in this twilight zone the evidential forces of the principle must be recognized, and while courts will go a long way in admitting expert testimony deduced from a well recognized scientific principle or discovery, the thing from which the deduction is made must be sufficiently established to have gained general acceptance in the particular field to which it belongs (8)'."

The National Safety Council, the hub of the safety movement in America, has advocated the use of chemical tests in combating the serious problem caused by the drinking driver. Although many methods for

the determination of ethyl alcohol have been thoroughly studied previous to publication, only a few have enjoyed limited acceptance by the public, courts, or workers within the field.

The National Safety Council Committee on Chemical Tests for Intoxication appointed a technical sub-committee** to evaulate the various methods of chemical analysis. Michigan State College, Department of Police Administration, not having previously participated in alcohol studies including methods of analysis, was selected to conduct the required research. The project was initiated in 1948 and work started in September of that year.

The accuracy of each method was first determined, where possible, by analysis of solutions of water, blood, and urine containing known concentrations of ethyl alcohol. The same determinations were then used for the analysis of body fluids obtained from human subjects who had consumed measured amounts of alcohol. A comparison was then made between blood, urine and breath to determine their correlation with reference to alcohol concentration.

** The sub-committee was formed in 1948. Dr. C. W. Muehlberger was appointed chairman.

## HISTORY

The past half century has seen a great accummulation of knowledge concerning the physiological aspects of alcohol. Since the beginning of the 20th century, many of the published methods have used potassium dichromate in the presence of sulfuric acid as the basis of ethyl alcohol determination to study its absorption, distribution and elimination of the animal body.

METHODS OF ANALYSIS:

The first attempt at colorimetric determination by dichromate oxidation of alcohol was made by Nicloux in 1900 (9) and is the source of reference for numerous succeeding methods. The unique principle which he employed was a direct volumetric determination of an alcohol-containing distillate. Standard dichromate solution was added, a drop or two at a time, to the collected distillate sample, heating and shaking the solution between additions until the color changed from yellow to green. The addition was continued until the end point was reached. An excess of dichromate was indicated when the color changed from blue-green to yellow-green. The amount of dichromate used was proportional to the amount of alcohol present in the sample, therefore the intensity of the color at the end point was also dependent on the amount of alcohol present. Shortcomings of the method were that the total volume at the end point was variable due to the amount of dichromate added and

that the color change was hard to detect.

Another method for the determination was also used in the latter part of the 19th century. The distillate was introduced into a standard amount of dichromate and sulfuric acid. This method was used by Bodlander(10) Benedict and Norris (11) and Pringsheim (12). Bodlander determined the degree of reduction of the dichromate by comparison with suitable standards. This method was later employed by Heise (13) in which permanent standards were made in the laboratory or purchased commercially. The other workers estimated the excess of dichromate by titration with ferrous ammonium sulfate. Pringsheim used a side spot test with ferricyanide towards the end of the titration. The formation of prussian blue indicated the end point. Benedict and Norris used an excess of ammonium sulfate and determined the excess by titration with potassium permanganate. The end point was a change from faint blue-green to red. This end point was later employed in a similar method by Southgate.(14)

A significant modification of the "Nicloux Method" was developed by Widmark (15). He followed the method of Bodlander and others except in the determination of the excess dichromate after oxidation of the alcohol sample. The excess of dichromate was reduced by the addition of a known amount of alcohol until a standard color was obtained. The weight of alcohol added was subtracted from the total amount required to reduce the dichromate. This value was equivalent to the alcohol con-

tained in the sample.

Muehlberger (16), Kozelka and Hine (17), and others preferred to use an iodometric determination for the excess of dichromate. The iodine formed by addition of potassium iodide to the dichromate acid mixture was titrated with sodium thiosulfate. A starch solution was used as the indicator and the end point was reached when the color changed from deep blue to light green.

RELATIVE CORRELATION IN BODY FLUIDS:

The earlier methods of analysis were used primarily for the detection of alcohol in the blood. Later studies showed a correlation between blood and urine alcohol values. The services of a physician or medical technologist were needed to obtain a sample of blood. Although urine samples usually cannot be taken at the scene of the accident or violation, they are readily obtainable without the need of a trained specialist. Nicloux (18) and subsequently Widmark (19) contended that the concentration of alcohol in the urine was the same concentration as that found in the blood. Studies on men by Miles (20) showed that venous blood did not parallel the alcohol concentration in the urine for the first two hours after the consumption of alcohol. Heise (21) pointed out that the alcohol content of the urine increased at a slower rate than that of the blood. The maximum concentration of blood alcohol was reached one hour after the ingestion of eight ounces of 100 proof whiskey; whereas in urine the maximum

concentration was reached after three hours. The relation between blood and urine alcohol was approximately 1:1.35 during the period when disappearance exceeded absorption. Southgate (14) reported the ratio of blood alcohol to urine as 1:1.37. Carlson and Muehlberger (22) arrived at a value of 1:1.32. This ratio would hold true only after a period of at least two hours had elapsed after the ingestion of alcohol. Any urine test previous to this time would give a lower value than that of blood. Haggard and Greenberg (23) working with dogs obtained urine samples from the pelvis of the kidney by catheterization and compared its alcohol concentration with that of arterial blood. The ratio they obtained by this method reflects the relative solubility of alcohol in blood and urine and was found to be 1:1.144. Three hours after the ingestion of alcohol they found that the alcohol content of arterial blood was approximately 5% higher than venous blood. From this data the ratio of venous blood alcohol to urine was shown to be approximately 1:1.22, a ratio which within the limits of experimental error approaches that found by Heise, Southgate and Carlson and Muehlberger.

The distribution of alcohol in the body was found by Harger, et al to be dependant on the water content of the tissue studied. After equilibrium was reached the brain alcohol concentration was used as a basis of reference and given a value of 1. The relative values for blood, liver, muscle and stomach contents were 1.18, 0.95, 1.01 and

1.13 respectively. This study showed that the alcohol concentration of the brain could be predicted from that found in blood.

In 1910 Cushny (24) pointed out that the distribution between alveolar air and the blood of volatile substances such as acetone, ether, and alcohol obeyed Henry's law, so that the concentration of alcohol in the blood could be predicted from the concentration in the alveolar air. The use of breath analysis as an indication of intoxication was first attempted by Bogen (25) in 1927.

Liljestrand and Linde (26) investigated the relationship between the alcohol content of arterial, venous and capillary blood following alcohol consumption and found them to be the same. The distribution coefficient of alcohol between air and blood at 37°C was found to be 0.00063 to 0.00073, and at equilibrium at 31°C one ml. of blood contained as much alcohol as 2 liters of air. A constant relationship between alcohol in the alveolar air and blood was also found by Haggard and Greenberg (23) but the value they obtained was quite different. Haggard and Greenberg reported a ratio of 1 to 1145 for dogs compared to Liljestrand's and Linde's figure of 1:2000 for humans. Harger (27) *et al* found values similar to those of Bogen, Liljestrand and Linde, and later also confirmed by Jetter and Forrester (28).

Fitzgerald and Haldane (29) in 1905 showed that the alveolar air of normal subjects contained about 5.5 per cent of $CO_2$ by volume. This

corresponded to 190 mg. of $CO_2$ contained in two liters of breath.

Harger et al (27) in 1938, conceived a way in which the relationship between blood and breath could be easily adapted to chemical tests for intoxication. The method somewhat simplified the procedure of analysis and eliminated the need of a trained medical person to obtain blood samples. A portable apparatus* was constructed by which results were obtained more rapidly than by any previous methods. Operators using the testing device were first schooled in its manipulation before using the apparatus in the field. Very few technics had to be mastered and a person with normal intelligence could qualify as an operator.

Harger found that when breath containing .175 mg. of alcohol was passed through one ml. of a N/20 solution of potassium permanganate in a 56 per cent solution of sulfuric acid, a certain color reduction occurred in the permanganate solution. By using this end point color it was possible to oxidize .175 mg. of alcohol by comparison with a blank. The difficulty with this blank was that complete reduction of the permanganate had occurred at the end point. Harger decided to use a blank so that when matched in color by the reduced permanganate, there would have been .169 mg. of alcohol oxidized. In this way any additional alcohol passing through the chamber would give more reduction of color, showing

* Drumkometer, manufactured by Stephenson Corp., Red Bank, New Jersey.

that the end point had been passed. This resulted in a more definite end point.

The breath was passed from the oxidation tube into a dehydrite* tube to remove any moisture. The moisture free breath was then passed through a previously weighed ascarite** tube. The amount of $CO_2$ which accompanied .169 mg. of alcohol could be determined. Normal expired air contains approximately 62.5 per cent alveolar air and from this the concentration of alcohol in alveolar air was determined. This value could in turn be converted to blood alcohol concentration by using the ratio of blood to alveolar air 1:2000. A set of conversion tables accompanies each instrument in which the alcohol concentration of blood can be found corresponding to the weight of $CO_2$ absorbed in the ascarite**tube. Analytical balances are necessary to weigh the ascarite tubes to obtain accurate weight to one tenth of a milligram.

Jetter and Forrester (28), 1941, recognizing the same correlation between blood and breath alcohol as Liljestrand and others, developed the perchlorate*** method for the determination of alcohol in expired

* Dehydrite - commercial preparation used for water absorption and obtained from Stephenson Corp.

** Ascarite - mixture of sodium hydroxide and asbestos used to collect $CO_2$ samples. Obtained from Stephenson Corp.

*** Intoximeter - manufactured by Intoximeter Association, Niagara Falls, New York.

air. The breath from the subject was passed through a train which contained magnesium perchlorate in the first chamber to remove moisture and alcohol. The expired air then entered a second previously weighed chamber in which the $CO_2$ was absorbed by ascarite. The train contained a U-tube connection so that the breath could be bypassed through a fritted glass tube containing a measured amount of permanganate and sulfuric acid. The rate of flow was calibrated so that the length of time for the reduction of the permanganate color could be used to estimate the concentration of alcohol in the blood stream. Complete loss of color in a period of 39 seconds or less indicated that the subject's blood contained 0.15 per cent. alcohol or more, 80 seconds corresponded to 0.05 per cent. The length of time for the reduction of color was indirectly proportional to the alcohol concentration found in the blood.

The advantage of the method of Jetter and Forrester was the compactness of the unit permitting it to be easily carried. The unit was charged with perchlorate, the ascarite tube weighed, and the unit sealed and signed out of a central laboratory. The sample of breath of a subject was passed through the apparatus and returned to the laboratory for analysis. A minimum number of specially trained personnel was required. A central laboratory with a trained chemist

could dispense and analyze the units for a relatively large area.

Each of the previous methods for analysis of breath has required trained technicians to insure accurate determinations. Greenberg and Keator (30) devised a chemical robot* which automatically determined and recorded the blood alcohol concentration by analyzing the breath. The operation of the unit was simplified so that a non-skilled operator merely had to follow directions to obtain reliable results. The basis for analysis was the liberation of iodine when breath containing alcohol was passed through a thermostatically controlled heated tube of iodine pentoxide. The free iodine was then passed into a fixed volume of starch and potassium iodide solution. The color developed by the starch-iodine complex was measured photo-electrically.

A sample of iodine pentoxide at a constant temperature and a constant flow of air yielded free iodine in proportion to the amount of alcohol oxidized. An ammeter connected to the photo-electric cell was calibrated empirically by passing known amounts of alcohol through the iodine pentoxide tube and noting the degree of deflection on the scale. The coefficient of distribution of alcohol between blood and alveolar air being known and a measured amount of expired air being used for each determination, the values for blood alcohol could

* Alcometer, manufactured by Alfred Bicknell Associates, Cambridge, Mass.

be directly read on the ammeter.

The Intoximeter and Drunkometer employed balloons for trapping the expired air from the subject. The receiving tube of the Alcometer was placed in a chamber heated to just above body temperature to prevent condensation. Haggard et al (31) had shown that when the environmental temperature was below the body temperature then condensation of moisture in a balloon caused a loss of alcohol due to its solubility in water. Harger (32) refuted these findings in showing that the time required to run the Drunkometer test would permit a drop of 6°C in the breath sample. The maximum error by this source was only 2 to 4 per cent lower than that found when the breath was maintained at 34°C, the temperature of expired air. Jetter and Forrester (28) made breath alcohol determinations in which the sample was collected in a warm room and also where the temperature was 15°C. They observed no difference in the results.

The Drunkometer and Intoximeter are manufactured commercially. The Alcometer is a relatively new apparatus in the field of chemical tests for intoxication. Although it has been used extensively by the Yale School*, it is yet to be sold commercially for use by law enforcement agencies.

SPECIFICITY OF CHEMICAL TESTS:

The question of specificity of chemical tests for intoxication

*Yale University, Laboratory of Applied Physiology, New Haven, Conn.

has been doubted by many workers. Florence (33) in 1922, stated that the Widmark method was not specific for measurement of alcohol. He recommended isolation and qualitative tests for alcohol. It was true that easily oxidizable organic substances introduced into hot acid dichromate solution would have been determined as alcohol. The question was to determine if these organic compounds existed in the blood stream in significant amounts and would be steam volatile during distillation and thus appear in the distillate. Such substances as ether, salicylic acid, chloral, chloroform, acetone and lactic acid were checked by Heise (34) for possible sources of error. Lactic acid and salicylates were not steam volatile so did not show up in the distillate. A sample of blood distilled from a patient who had undergone ether anesthesia for one hour showed a test of 0.002 per cent. According to Bavis and Arnholt (35) chloral hydrate is fatal in a 5 gram dose. A solution equivalent to a concentration of 0.1% or 1 mg. per ml. of body fluid for a man of 150 pounds would be about 45 grams, hence the utter impossibility of chloral hydrate as a source of error. Bavis and Arnholt found that no organic substance whose solubility in water was less than one milligram per ml. could be found in the urine in higher concentrations. Water soluble solids, unless they are steam volatile such as chloral hydrate would not be found in the urine. All clinically

possible substances are, weight for weight, more toxic than ethyl alcohol, with the exception of acetone which is infinitely soluble, relatively non-toxic, is excreted by the kidneys and is steam volatile so would appear in the distillate. Purves-Stewart (36) reported that a level of 30 to 50 mg. of acetone per 100 ml. of blood was highly dangerous to life with survival rare at 35 mg. per 100 ml. In actual practice, therefore, a person would have to be comatose before reaching a significant concentration of acetone in the blood to give a false result.

If one applies the critera of chemical possibility, clinical possibility and practical possibility, one may easily prove that in the practical use of the test, no substance but ethyl alcohol would be found in the distillate in sufficient quantities to give any significant results.

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## EXPERIMENTAL

Alcohol introduced into the human body quickly diffuses throughout the whole body and distributes itself in proportion to the water content of the various tissues. If, after equilibrium is reached, a measurement of the alcohol content can be made in a body tissue or fluid, the concentration in other tissues may be estimated.

The center which is effected most by alcohol is the brain. The ultimate goal in the measurement of intoxication is to determine how much alcohol is contained in the brain, but it is obviously impractical to remove a portion of brain tissue for analysis. The determination of the alcohol content of the blood has been used for the estimation of the alcohol concentration of other body fluids, organs, or tissues because the circulatory system reaches all parts of the body and maintains an equilibrium with the tissues.

The reliability of the method for determining the alcohol content of blood had to be established in order that it could be used as a valid test for intoxication. Quantitative oxidation of alcohol in dichromate was checked by introducing known amounts of alcohol into the acid dichromate solution. Initially water solutions of alcohol were distilled into tubes of acid dichromate.. Subsequently body fluids containing known amounts of alcohol were prepared and analyzed by the same procedure. Chemically pure reagents and triple distilled water were used to reduce possible errors from extraneous sources. National Bureau of Standard pipettes were available for taking sample aliquots

and transferring standard solutions.

The first phase of the study was to become familar with procedures and technics of analysis. Most of the methods of analysis employed iodometric titration for the determination of excess dichromate after oxidation of the alcohol was complete. A number of sample titrations were performed to become acquainted with the end point and to check the accuracy of the method. Solutions used were those employed in Muehlberger's (16) method for alcohol determination.

Determination of Alcohol in Water-Alcohol Solutions of Known Concentration.

In order that analysis could be made without possible interference from reducing compounds in body fluids, it was decided that analysis of water-alcohol solutions would first be made. Constant boiling alcohol (B.P. 78°C) was prepared. The per cent of alcohol by weight was determined by gravimetric procedure using a 25 ml. specific gravity bottle and comparing to water at 20°/20°C. The specific gravity was then converted to per cent of ethyl alcohol and found to be 92.12% by weight. The alcohol was sealed in two ounce sample bottles until needed for use in preparing known solutions.

Water-alcohol solutions of known concentration were prepared by transferring alcohol (92.12%) from a weighing pipette into a volumetric flask and diluting to a specific volume. Calibration of the pipette enabled one to approximate the concentration of the solution desired.

The exact composition was determined by the difference in the weight of the pipette before and after delivery.

$$\frac{\text{Wt. of alcohol delivered} \times 92.12\%}{\text{(Total wt. of solution)}} = \% \text{ alcohol by wt. w/w}$$

Errors in the determination of alcohol may arise from one of the following sources; loss of alcohol through distillation, incomplete oxidation by the acid-dichromate, or loss of volatile acetaldehyde before complete oxidation has taken place. If an excess of potassium dichromate and sulfuric acid are present the reaction should go to completion according to the following equation.

$$2K_2Cr_2O_7 + 8H_2SO_4 + 3C_2H_5OH \longrightarrow 3CH_3COOH + 2Cr_2(SO_4)_3 + 2K_2SO_4 + 11H_2O$$

Known alcohol solutions were pipetted directly into the acid-dichromate solution to insure that quantitative oxidation of the alcohol to acetic acid was demonstrable. The strength of the potassium dichromate solution was 0.434N, the same as used by Muehlberger. 5 ml. of this solution was equivalent to 25 milligrams of alcohol if completely reduced. Oxidation was carried out in a covered pyrex test tube (1 in. by 6 in.) in a boiling water bath for fifteen minutes. The extent of reduction of the potassium dichromate was determined iodimetrically. The free iodine liberated upon the addition of potassium iodide to the acid-dichromate was titrated with sodium thiosulfate. A one per cent water solution of soluble starch was used as the indicator. The number of milliliters of $Na_2S_2O_3$ used in the sample titration was taken as S.

$K_2Cr_2O_7 + 6KI + 7H_2SO_4 \rightarrow 3I_2 + 4K_2SO_4 + Cr_2(SO_4)_3 + 7H_2O$

$3I_2 + 6Na_2S_2O_3 \rightarrow 3Na_2S_4O_6 + 6NaI$

Approximate 0.05N $Na_2S_2O_3$ was used in the titration. A blank titration was made to determine the exact amount of sodium thiosulfate equivalent to 5 ml. of 0.434N potassium dichromate. The milliliters of $Na_2S_2O_3$ for the blank titration was taken as B. The amount of alcohol oxidized was then calculated.

$\frac{B - S}{B} \times 25$ mg. = mg. of ethyl alcohol

$\frac{\text{gm. of ethyl alcohol} \times 100}{\text{wt. of sample}}$ = per cent by wt.

TABLE I

| % Alcohol in known | Average % Alcohol | Number of Detns. | Average % Recovery |
|---|---|---|---|
| .116 | .115 | 1 | 99 |
| .147 | .147(.145-.150) | 9 | 100 |
| .174 | .167(.165-.169) | 3 | 96 |

The next step was to take known samples and distill the alcohol to ascertain that there was no mechanical loss through distillation.

The method of distillation employed by Muehlberger involved the distillation of the alcohol from a kjeldahl flask through a steam trap into a Liebig condenser. The distillate was collected in a test tube containing the acid-dichromate mixture. A direct flame was used for heating the water and urine-alcohol solutions, whereas a steam

generator was employed in the case of blood. The same procedure for recovery of the alcohol from samples was used in the Heise(13) method. The degree of reduction of the potassium dichromate was found by comparing it with commercially prepared standards which were made up by adding a measured amount of alcohol to the acid-dichromate solution, e.g. 0.5 ml. of 0.1 per cent alcohol solution to a tube containing 3 ml. The tube was then placed in a boiling water bath for 5 minutes and the opening of the tube sealed by heating. This tube was then labeled as 0.05 per cent.

The modified Southgate (37) method removed the alcohol from the sample by aeration. The Cullen and Van Slyke urea apparatus was used and a steady stream of air was bubbled through the sample to transfer the alcohol into the acid-dichromate solution. The sample was placed in the first tube. The second tube contained a reagent consisting of sodium hydroxide, potassium and silver nitrate which removed the volatile reducing materials, e.g. acids and aldehydes. Into the third tube was introduced 10 ml. of 0.0868 N. potassium dichromate and 10 ml. of concentrated sulfuric acid. The dichromate solution was equivalent to 10 mgs. of alcohol when completely reduced. Titration of the excess of potassium dichromate after the oxidation of the alcohol was accompl-

ished by the same procedure as employed by Muehlberger except that a more dilute solution of sodium thiosulfate was used.

Table II contains data from the determination of known water-alcohol solutions.

Determination of Alcohol in Urine-Alcohol Solutions of Known Concentration.

Urine samples were collected from persons who had not consumed alcohol for at least four days previous to the collection of the sample. The specific gravity of various samples was checked by a urinometer and the average Sp. G. at 20°C was 1.02 (1.014 to 1.030). Data concerning alcohol concentrations in body fluids has been calculated by most observers without regard to their specific gravity. Whole blood has a specific gravity of approximately 1.060 (38). Alcohol concentrations calculated on a weight basis would therefore average 2% lower for urine and 6% lower for blood than if calculated on a weight/volume basis. All blood and urine values in this paper were calculated as the weight of alcohol found in a given weight of sample and reported as per cent.

The urine-alcohol solutions were prepared by the same method as used with the water-alcohol samples. Blank determinations were made to measure the reducing substances found in the unpreserved urine. Twenty-four hours after collecting the samples, the average per cent of

reducable material calculated as ethyl alcohol amounted to 0.003 per cent. By the end of one week the value increased to 0.011 per cent.

Table III contains data from the determination of known urine-alcohol solutions.

Determination of Alcohol in Blood-Alcohol Solutions of Known Concentrations.

Bovine blood containing 1% sodium oxalate as an anticoagulent was used for the preparation of the known blood-alcohol samples. A saturated solution of picric acid was added on the basis of 1 ml. of picric acid per ml. of blood at the time of distillation to precipitate the protein and prevent foaming. A blank determination was also made on the picric acid and the value obtained was added to the ml. of sodium thio-sulfate required for the sample titration.

Steam distillation of the blood sample was used in the methods of Muehlberger and Heise. No modification of the Southgate method was necessary. Direct heating of the blood samples has been used by Heise but a longer time was required than for steam distillation.

Table IV contains data from the determination of known blood-alcohol solution.

Determination of Alcohol in Body Fluids obtained from Human Subjects.

TABLE II — DETERMINATIONS OF KNOWN WATER-ALCOHOL SOLUTIONS

| % Alcohol in known | MUEHLBERGER | | | HEISE | | | SOUTHGATE | | |
|---|---|---|---|---|---|---|---|---|---|
| | Ave. % Alc. Detn. | No. of Detns. | Per cent Recovery | Ave. % Alc. Detn. | No. of Detns. | Per cent Recovery | Ave. % Alc. Detn. | No. of Detns. | Per cent Recovery |
| .10 | | | | | | | .11(.10-.12) | 5 | 110 |
| .12 | .11 | 3 | 92 | | | | | | |
| .13 | .12 | 4 | 92 | | | | | | |
| .15 | .14(.13-.14) | 20 | 93 | | | | | | |
| .16 | .15(.14-.16) | 18 | 94 | .16 | 7 | 100 | .16(.16-.17) | 3 | 100 |
| .17 | .17 | 6 | 100 | .18 | 5 | 108 | .17 | 1 | 100 |

TABLE III — DETERMINATIONS OF URINE-ALCOHOL SOLUTIONS

| % Alcohol in known | MUEHLBERGER | | | HEISE | | | SOUTHGATE | | |
|---|---|---|---|---|---|---|---|---|---|
| | Ave. % Alc. Detn. | No. of Detns. | Per cent Recovery | Ave. % Alc. Detn. | No. of Detns. | Per cent Recovery | Ave. % Alc. Detn. | No. of Detns. | Per cent Recovery |
| .12 | .12(.11-.12) | 7 | 100 | | | | | | |
| .15 | .14(.14-.15) | 5 | 93 | | | | | | |
| .17 | .17(.16-.17) | 3 | 100 | .16 | 6 | 94 | .16 | 1 | 94 |
| .20 | .18 | 5 | 90 | .18 | 4 | 90 | .18(.16-.19) | 3 | 90 |

TABLE IV — DETERMINATIONS OF BLOOD-ALCOHOL SOLUTIONS

| % Alcohol in known | MUEHLBERGER | | | HEISE | | | SOUTHGATE | | |
|---|---|---|---|---|---|---|---|---|---|
| | Ave. % Alc. Detn. | No. of Detns. | Per cent Recovery | Ave. % Alc. Detn. | No. of Detns. | Per cent Recovery | Ave. % Alc. Detn. | No. of Detns. | Per cent Recovery |
| .13 | .12(.12-.13) | 3 | 92 | .12(.12-.13) | 3 | 92 | .12(.12-.13) | 3 | 92 |
| .18 | .17(.15-.17) | 11 | 94 | | | | .16(.14-.19) | 4 | 89 |
| .19 | .17(.16-.17) | 9 | 90 | .18(.18-.19) | 3 | 95 | .18 | 3 | 95 |
| .23 | .19(.18-.20) | 6 | 83 | .20 | 4 | 88 | | | |
| .26 | .24(.23-.24) | 3 | 92 | | | | | | |

Human subjects were used to study the reliability of various chemical tests for ethyl alcohol intoxication with emphasis on the comparison of breath analysis with blood and urine. Men were used in 95% of the cases because they contribute about 95% of the traffic violations involving alcohol.

Subjects were chosen at random. The testing period began at seven p.m. and lasted until approximately midnight. Subjects were usually taken in groups of four, and an attempt was made to simulate the conditions of an evening of social drinking. The drinks consisted of one and one half ounce of 100 proof whiskey* taken either straight or mixed according to the desires of the particular subject. When mix was added, the drink had a final concentration of alcohol of about 20%. Each subject used his own discretion as to how many and how fast the drinks were consumed. During the evening, snacks of cheese, crackers, peanuts, popcorn and etc., were available for the subjects.

Urine samples were taken throughout the evening and the total volume of elimination was measured. Frequent breath samples were taken and the subjects were required to refrain from drinking at least ten minutes before the test was performed. This prevented any residual alcohol of high concentration in the tissue of the mouth from

*Kentucky Tavern. Bottled in bond by the Glenmore Distillers Company Owensboro, Kentucky. The whiskey was all taken from the same vat so that standard composition was received, and found to contain 41.14 % alcohol by weight. The liquor was checked by a whiskey hydrometer at 16°C and a reading of 100 proof was obtained.

contributing to a sample of expired air. Breath was analyzed by methods of Harger (27), Jetter & Forrester (28), and Greenberg and Keator (30). One or two blood samples were taken from each subject and placed in prepared vials containing preservative.

Transportation was furnished so that subjects could not drive their cars and were asked to refrain from driving until the effects of intoxication, if present, had disappeared.

Urine and blood specimans were refrigerated, and the subsequent analyses were completed as rapidly as possible, and by as many methods as sample size and time allowed. The ascarite tubes used in the Drunkometer and Intoximeter were usually weighed within 24 hours after the tests had been performed.

The Muehlberger method was used as a reference method in this study. The degree of accuracy was established by primary analysis of a number of known solutions. In the case of body fluids 5 or 10 ml. samples could be used depending on the amount of alcohol present. When the sample contained more than 1.5 mg. of alcohol per gram of fluid, 5 ml. was taken. The use of a large sample provided more reliable sampling and errors would not be magnified as much as in the case of the 1 or 2 ml. samples employed in most methods. The Heise method was as rapid as the Muehlberger method and less expensive to perform;

however it required pipetting 4 different solutions of various volumes, the largest amount being 2 ml. Moreover exactly 10 ml. of distillate had to be collected. The Muehlberger method required only 3 accurate measurements, a 5 ml. sample of body fluid, 5 ml. of the standard potassium dichromate solution, and the indirect determination of the excess dichromate. The strength of the sodium thiosulfate was approximately 0.05 N so that about 40 ml. was equivalent to 5 ml. of the potassium dichromate solution. Thus it was felt that any error was minimized by using the Muehlberger method.

The blood samples taken from the subjects were analyzed by the Heise, Southgate, and Muehlberger methods. The values obtained are shown in Figure 1 which is a comparison between the per cent of alcohol determined by the Muehlberger method and per cent alcohol found by the other two methods. Figure 2 shows the relationship between the values obtained in the analysis of blood by the Heise and the Southgate methods. Two identical values on the graph are indicated by the symbol o', three, by o'' and four by o'''.

Blood and breath samples were obtained from the subject within a fifteen minute period. The blood alcohol level was calculated from the value found by analysis of the breath using the Drunkometer. The blood sample was placed in a vial containing sodium fluoride calculated to supply at least 10 mg. per ml. of blood. When a combination of

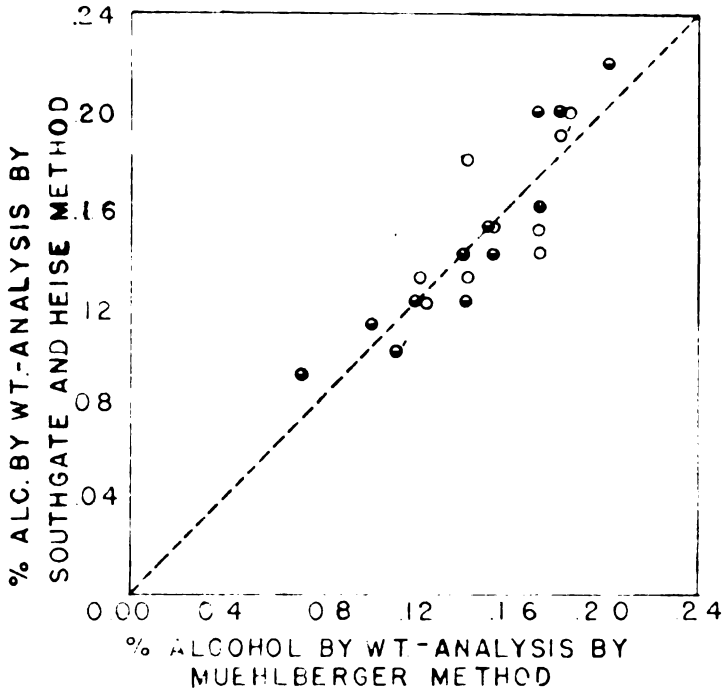


FIGURE 1 - COMPARISON OF BLOOD ALCOHOL CONC. BY DIRECT ANALYSIS

o - HEISE ANALYSIS
● - SOUTHGATE ANALYSIS

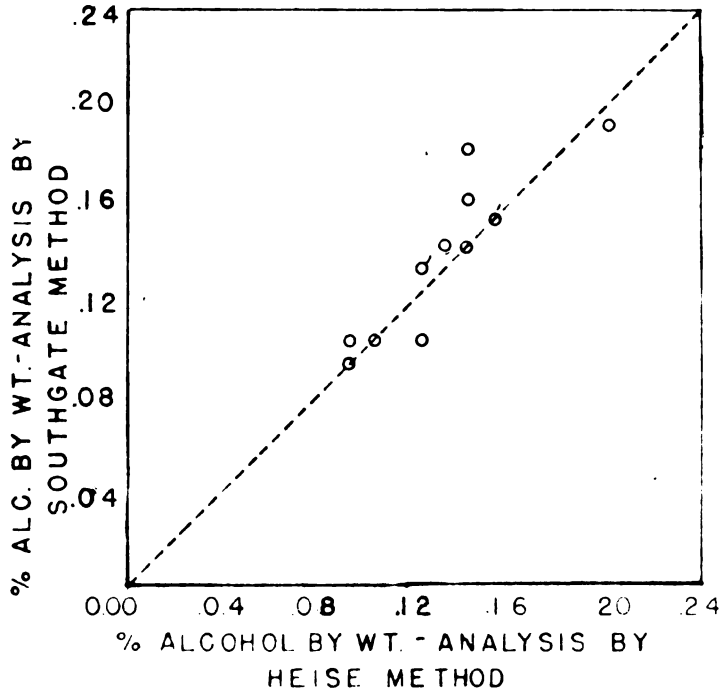


FIGURE 2- COMPARISON OF BLOOD ALCOHOL CONC. BY DIRECT ANALYSIS

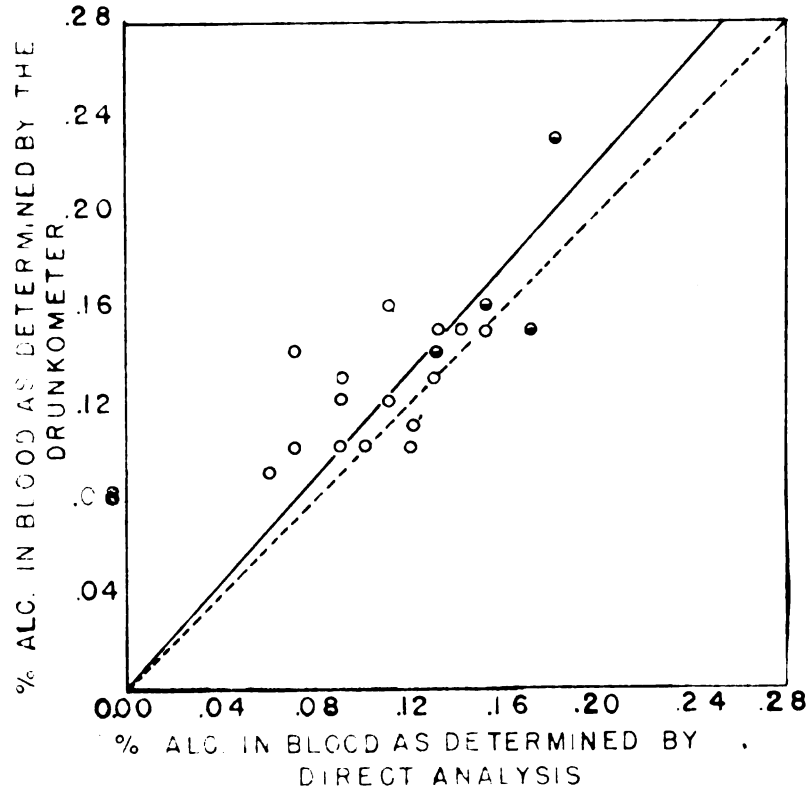


FIGURE 3 - COMPARISON OF BLOOD ALC. CONC. FOUND BY BREATH ANALYSIS AND DIRECT ANALYSIS OF BLOOD

o- SODIUM FLUORIDE PRESERVATIVE
●- SODIUM FLUORIDE PLUS SODIUM OXALATE PRESERVATIVE
R=0.982 T=22.17

sodium fluoride and sodium oxalate was used, the final concentration of each was at least 5 mg. per ml. of blood. Direct analysis of the blood by the Muehlberger method permitted comparison with the alcohol level as determined indirectly by breath analysis.

In Figure 3 the per cent by weight of the alcohol in the blood is compared to the per cent by weight calculated from the analysis of the breath by the Drunkometer.

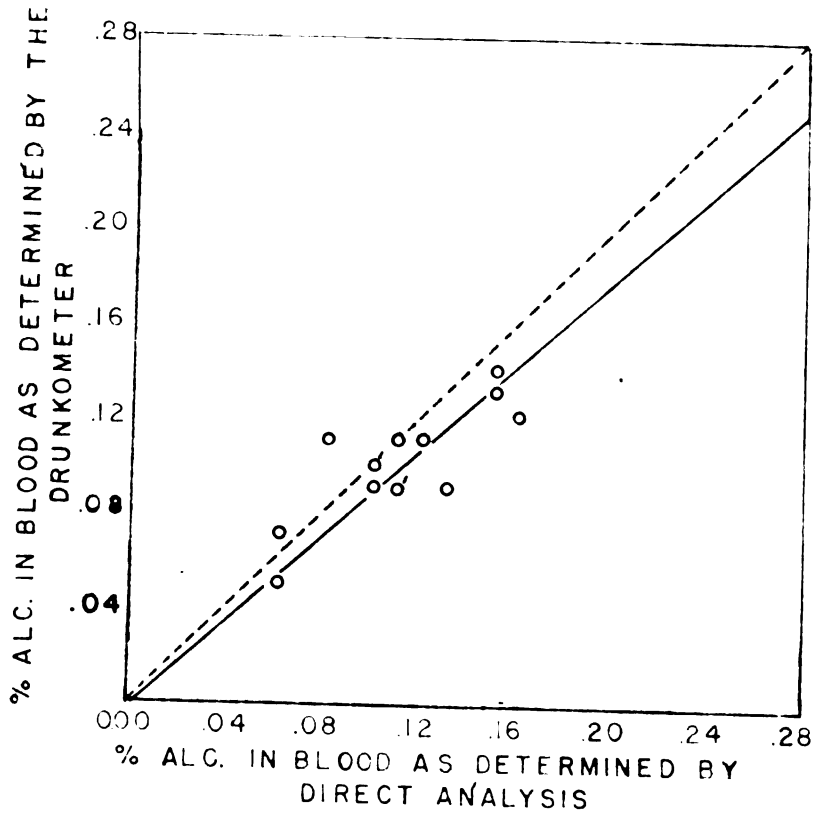


FIGURE 4 - COMPARISON OF BLOOD ALC. CONC. FOUND BY BREATH ANALYSIS AND DIRECT ANALYSIS OF BLOOD

o - SODIUM FLUORIDE PLUS SODIUM CITRATE PRESERVATIVE

R=0.988 T=27.69

Similar tests were performed using a mixture of sodium fluoride and sodium citrate as the preservative in the blood sample. The values obtained are found in Figure 4.

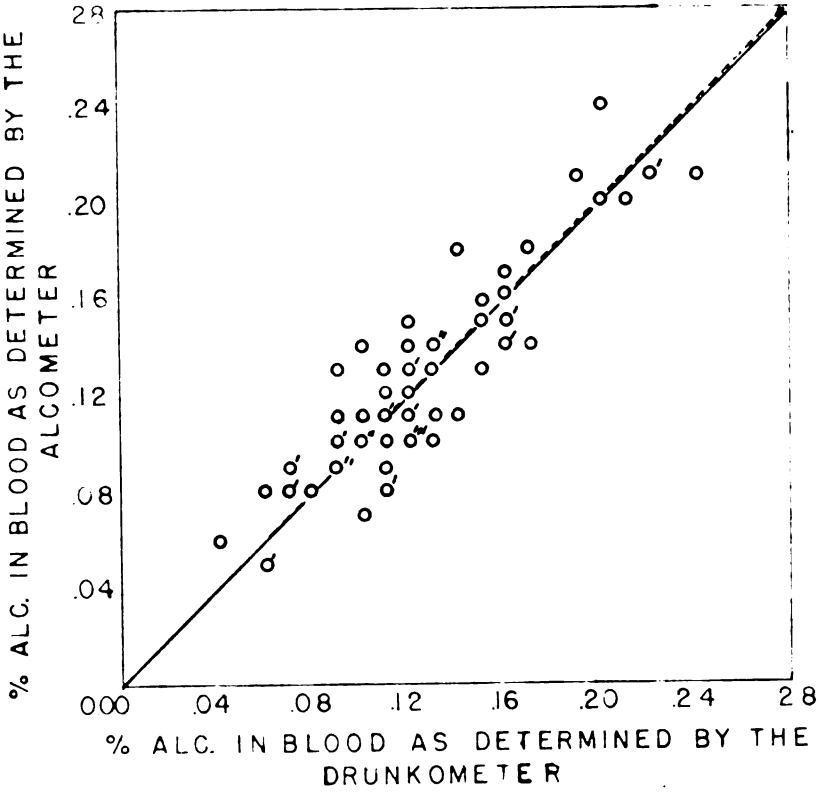


FIGURE 5- COMPARISON OF BLOOD ALC. CONC. FOUND BY BREATH ANALYSIS USING TWO DIFFERENT METHODS

R=0.990 T=54.22

Frequent breath samples were obtained from the subject. The alcohol content of the blood was determined by the Drunkometer, Intoximeter, and the Alcometer. Figure 5 shows the relationship between the values obtained by simultaneous tests using the Drunkometer and Alcometer.

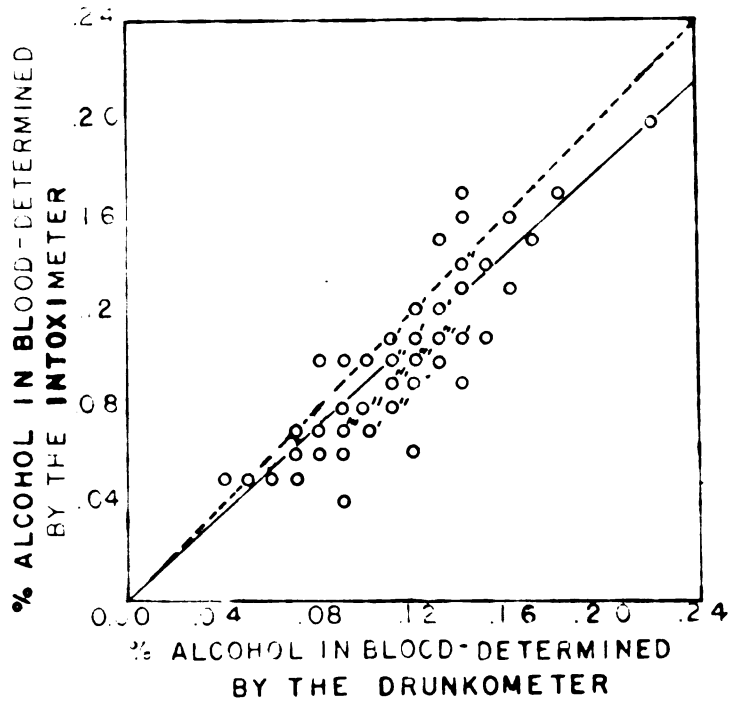


FIGURE 6-COMPARISON OF BLOOD ALC. CONC. FOUND BY BREATH ANALYSIS USING TWO DIFFERENT METHODS

R=0.993 T=53.26

A comparison of the values obtained by the Intoximeter is shown in Figure 6.

In the case of some of the subjects urine samples were obtained within 15 minutes of the time the blood samples were drawn. The urine samples were separated into two groups. The first included those samples which were passed either during the period of consumption of alcohol or less than one hour after the last drink of liquor had been given. The blood and urine alcohol values are presented in Figure 7. The second group of urine samples included those that were passed at least one hour after the last drink had been consumed. The relation between the alcohol content in urine and blood at a stage near equilibrium in the body is shown in Figure 8.

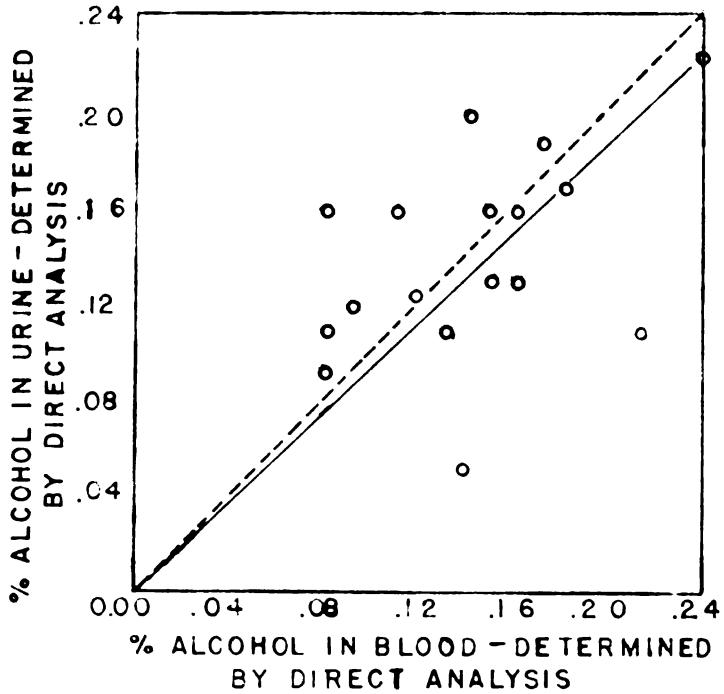


FIGURE 7- COMPARISON OF THE ALC CONC. IN BLOOD AND URINE

R=0.924 T=9.24

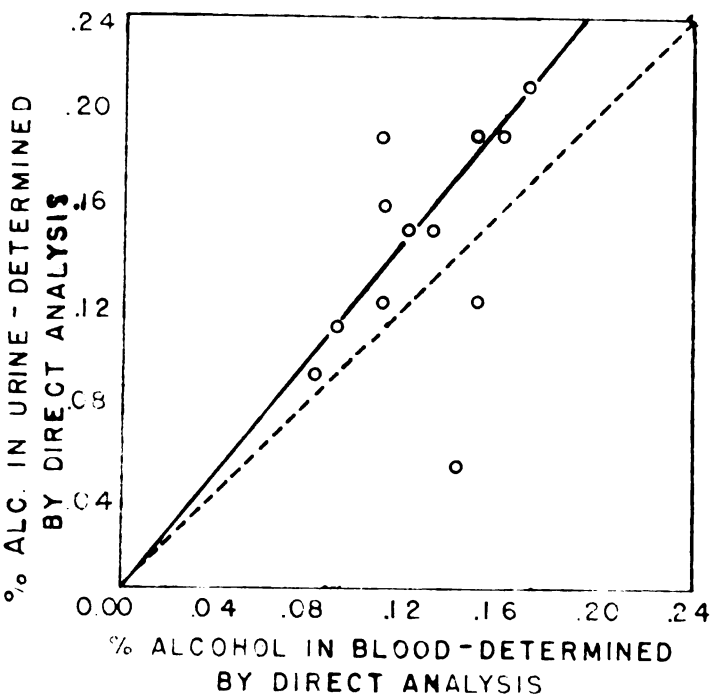


FIGURE 8- COMPARISON OF THE ALC. CONC. IN BLOOD AND URINE

R=0.992 T=7.41

## DISCUSSION AND CONCLUSIONS

The reliability of three methods of analysis, the Muehlberger, Heise, and modified Southgate methods, was established by the determination of alcohol in known solutions. Quantitative results could be obtained when the water-alcohol solution was pipetted directly into the acid-dichromate. When alcohol was distilled from the samples, lower values were obtained as shown by the per cent of recovery from water, urine, and blood-alcohol solution. The average recovery obtained in the analysis of blood-alcohol solutions was 90%, 91%, and 92% respectively by the Muehlberger, Heise and modified Southgate methods.

A comparison was made between the per cent of alcohol found by direct analysis of the blood and the calculated values from the analysis of the breath and urine of human subjects. When sodium fluoride was used as an anticoagulant and preservative, the mean value obtained by breath analysis of the blood was higher than the mean value obtained by the direct analysis of the blood. The inability to obtain good correlation may have been due to the partial clotting of the blood. When the blood clot was broken up, air bubbles in the sample made accurate measurements with a pipette impossible. A preservative consisting of sodium fluoride and sodium citrate prevented clotting of the blood and a more uniform correlation between

blood and breath was obtained.

Statistical analysis of the data by the usual methods gave values for R (coefficient of correlation) and T (significance of the correlation coefficient). The regression line was determined by the method of least squares.

The regression line for the comparison of blood and breath analysis showed that direct analysis of blood preserved by fluoride and citrate gave a higher value than that calculated from breath analysis by the Drunkometer (1:0.988).

The ratio of the mean values obtained by the blood alcohol estimation by the Drunkometer and Alcometer was 1:0.999. The values found for R and T indicate that comparable results may be obtained with the Alcometer and Drunkometer if used under similar conditions. The Intoximeter, in most cases, gave lower results than the other methods used for the analysis of the breath.

Urine was not satisfactory for estimating the blood alcohol concentration when the subject had been drinking less than one hour before the urine was passed. The correlation between the blood and urine alcohol values depends on the degree of equilibrium that has been attained in the body. The alcohol content of urine samples obtained at least one hour after the last drink showed a definite correlation with the blood alcohol level. Under these conditions the

ratio of blood-alcohol to urine-alcohol was 1:1.24. The ratio of 1:1.32 obtained by Heise and others indicates that more than one hour is necessary after the consumption of alcohol for the establishment of equilibrium between the blood and urine alcohol.

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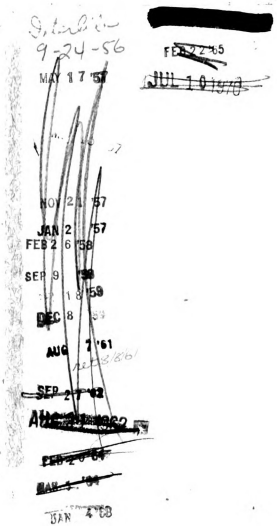
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