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Kurt M. Dubowski
Iowa Department of Public Safety

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Some Practical Laboratory Aspects of Forensic Alcohol Determinations

By Kurt M. Dubowski

Determination of ethyl alcohol in human biological material is the most common forsenic chemical examination, the majority of such determinations being performed in connection with investigations to establish the presence or absence of alcoholic influence in operators of motor vehicles. Utilization of alcohol determinations by law enforcement agencies in the United States continues to increase rapidly as demonstrated by an increase of 220% in the number of cities employing chemical tests for alcoholic influence during the last 4 years[1]. Twenty-three states now have chemical test legislation. Because of the rapidly expanding employment of forensic alcohol determinations, it seemed pertinent to examine some practical laboratory aspects of such tests in the light of our experience with approximately 25,000 such examinations during the past ten years.

I. Source, Type, Choice and Quantity of Specimens

Source, Type and Choice of Specimens

The great majority of forensic alcohol determinations are performed on specimens of blood, breath, and urine from motor vehicle operators or pedestrians, with occasional analyses of other body materials in fatalities. Since alcoholic influence, in those twenty-three of the United States having statutory definitions, is defined in terms of the concentration of ethyl alcohol in the blood, it seems most direct and legally most acceptable to analyze blood specimens. Practicability considerations, such as unavailability of persons legally or technically qualified to obtain blood samples, the necessity for securing specimens in the field, or other local conditions, however, frequently make it desirable or necessary to use specimen materials other than blood. Breath and urine specimens are the most frequently employed under such conditions; each again having certain advantages and shortcomings. Obtaining a urine specimen from a female subject requires separate physical facilities and a female attendant in order to be able to identify the specimen as to its origin, non-contamination or dilution at a later time, perhaps in court. Breath specimens are not readily collectable in duplicate or in sufficient quantity for separate later replicate analyses by systems other than the Intoximeter (which makes such duplicate sampling an expensive and cumbersome procedure), and must be analyzed very soon after collection for reliable results. In fatal traffic accident victims not coming to autopsy, it may be difficult to obtain any specimen material, blood being usually the most readily and consistently available substance.

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While many law enforcement agencies routinely obtain blood specimens without difficulty, it should be understood that the obtaining of a blood specimen by any penetration of the body constitutes, in some jurisdictions, practice of medicine and may be carried out only by a licensed medical practitioner or, by certain specified persons, under his immediate direction. In New Jersey, the "taking of a blood specimen from a living human being" has been declared to be the practice of medicine[2], and the statutory exemptions while including professional nurses operating in each particular case under the specific direction of a regularly licensed physician do not include medical technologists or similar persons[3]. The recently enacted implied consent chemical test law of the State of New York specifically provides that "only a duly licensed physician acting at the request of a police officer may withdraw blood for the purpose of determining the alcoholic content therein"[4]. There have also been recent contrary court decisions. The Supreme Court of Idaho held that taking of a blood specimen for alcoholic analysis by a laboratory technician did not constitute practice of medicine[5]. The Supreme Court of North Carolina approved the taking of a blood specimen from a defendant by a chemist[6]. Other states have similarly varying provisions and statutory limitations upon the drawing of blood specimens from living persons; and while some laxness in the enforcement of these provisions exists in many jurisdictions, it appears entirely possible that criminal proceedings could be instituted against anyone not authorized by statute to obtain blood specimens upon his acknowledging such action in court in the course of routine testimony concerning the origin and identity of a specimen. Whether testimony concerning the results of analyses upon specimens thus obtained by legally unqualified persons would be admissable in the several jurisdictions is an interesting speculation. Similar legal restrictions concerning the procurement of breath and saliva, and urine from living persons have not yet been enacted although many jurisdictions do have rigid limitations upon the postmortem removal of any body material.

In the use of blood specimens for routine alcohol determinations a number of technical problems must be considered. Foremost among these is the type and donor site of the blood specimen, since it is well known that at different stages of absorption the concentration ratio of arterial and venous blood alcohol varies. It has therefore been suggested that only venous blood alcohol analyses form the basis of precise interpretations, and venous blood is probably also the most satisfactory for ease of obtaining adequate quantities. Capillary blood specimens, aside from any alcohol level

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variation attributable to non-existence of distribution equilibrium, are also subject to various errors of collection technique during the sampling, such as dilution with tissue juice, and proportionately greater relative measurement errors because of the smaller absolute quantities sampled.

There has been some controversy over the relative alcohol levels in blood plasma, blood serum and whole blood; and a number of investigators have claimed various plasma/whole blood and serum/whole blood ratios greater than 1.00[8-11]. Although the several divergent ratios tend to be more uniform if calculated on an identical weight/volume basis, it is simple to circumvent this problem entirely by utilizing whole blood exclusively, a sounder procedure from both the analytical and the physiological points of view. Whole blood from living subjects is readily treated to prevent both coagulation and change of original alcohol contents. In any large scale testing program, however, occasional blood specimens are received which are partially clotted through use of inefficient anticoagulants or because of failure to mix the blood thoroughly with the preservative after drawing. Such clotted specimens can be analyzed without difficulty. Among suggested techniques for analysis of partially clotted blood specimens is that of Crosby, who obtains a homogeneous mixture of serum and clot by passing the mixture through sterile cloth[12]. Our preferred routine is to obtain a liquid blood homogenate by using a Pyrex tissue grinding vessel with Teflon pestle* operated either by hand or slow speed stirrer, and then to draw an aliquot of the homogenate into the sample pipette through a Nylon or monel-metal filter such as the disposable blood filters supplied with blood transfusion recipient sets†.

Urine alcohol analyses are widely employed by law enforcement agencies and clinical laboratories for the indirect determination of the blood alcohol level. Aside from any practical collection facilities problem, two vital technical considerations are encountered, one being the question of the true instantaneous ureteral urine/venous blood alcohol ratio at the time of excretion, and the other being a time relationship problem since any single random bladder urine specimen represents a pooled ureteral urine sample, possibly further altered to some extent by alcohol diffusion through the

*Tissue grinder, size A (10 ml.), Cat. No. 4288-B, Arthur H. Thomas Co., Philadelphia 5, Pa.

†Sterile disposable blood filter, monel metal, List No. 4427. Abbott Laboratories, North Chicago, Illinois.

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bladder mucosa [13,14]. Consideration of the former question is beyond the scope of this discussion; but the latter poses a practical sampling problem. Neglecting the effect, if any, of alcohol diffusion through the bladder wall since this cannot be controlled in sampling, it is still necessary to employ a urine sampling technique which will allow a valid estimate of the ureteral urine alcohol concentration at a known time; which can then, in turn, be converted to blood alcohol level by dividing by the urine/blood alcohol ratio, for which the most commonly accepted values are 1.30 to 1.35. Most of the valid sampling systems are based upon the technique suggested by Haggard et al. who recommended that the bladder be emptied and that one-half hour later the subject again be requested to void; the second sample being analyzed and the analysis value divided by 1.3 being taken as the concentration of alcohol in the blood at the time the test was made[7, 13]. The New York State Police Scientific Laboratory routine includes obtaining an additional urine specimen one and one-half hours after the first emptying of the bladder[15]; and our preferred routine is to collect two urine specimens at precisely known intervals of 30 to 45 minutes after initial complete emptying of the bladder, analyzing both specimens in order to determine whether the subject's blood alcohol level, if any, was increasing or decreasing during the sampling period. In estimating blood alcohol levels from urine specimens thus collected, one must realize that the alcohol concentration of the second, third, and later specimens after initial emptying of the bladder actually represents an average of an infinite number of instantaneous ureteral urine alcohol levels during the entire secretion period of that specimen; and that consequently the blood alcohol level calculated by use of the solubility ratio factor is that at a time exactly midway between the immediately preceding emptying of the bladder and collection of the specimen being analyzed, and not the level at the time of any one voiding.

These procedures leave one possible question of validity open, since they assume that the second or later specimens represent a pool of ureteral urine collected only since the immediately preceeding emptying of the bladder: How can the sampler or analyst be certain that the bladder was actually emptied completely, and that no residual urine was retained either deliberately or because of factors not under the control of the subject? It seems possible that the inability to answer this question categorically might be legally disabling to the introduction of testimony based upon any of the collection procedures outlined. That the timed-interval collection technique, nevertheless, is far superior to the attempted estimation of blood alcohol levels from single random pooled bladder urine is shown in Table I by several examples from our laboratories which illustrate the range of possible errors.

Table I

Correlation of Antemortem Blood and Random Urine Alcohol Levels.

Case	Random Urine Alcohol, %W/V	Blood Alcohol %W/V	Urine/Blood Alcohol Ratio	Calculated Blood Alcohol, %W/V (U/B = 1.30)
1	0.32	0.43	0.75	0.24
2	0.28	0.19	1.47	0.21
3	0.25	0.15	1.66	0.19

A final factor in urine alcohol analyses may be mentioned in passing. Since alcohol passes from the blood to the urine by diffusion, it is distributed according to the relative water content of the two fluids. While the water content of blood, as shown by its specific gravity, is fairly constant that of urine is known to vary considerably. It is to be expected, therefore, that the urine/blood alcohol ratio would decrease with increasing specific gravity of urine; and this fact has been experimentally observed by Moritz and Jetter who reported that the ratio decreases by approximately 0.07 for each urine specific gravity increment of 0.010 between 1.010 and 1.040[14], and by Haggard et al. who reported a somewhat smaller rate of ratio decrease[13]. It is obvious that in most forensic urine alcohol analyses these differences can be neglected.

Postmortem Specimens

In alcohol determinations on postmortem specimens, rigorous precautions are necessary to safeguard the validity of the results. Precautions are required not only to prevent contamination of samples, but also to minimize in vitro changes of the original alcohol level after removal from the body. It has been generally recognized that enzymatic oxidation of alcohol to acetic acid can and does occur postmortem in the liver both before and after its removal from the body[16-19], but it is less generally realized that alcohol can also be oxidized to acetaldehyde and acetic acid in vitro by brain, kidney, heart and other tissues.[16, 19-21] Since the oxidation is an enzymatic process, it can be minimized in vitro by effective refrigeration, preferably at –20°C., which also tends to minimize putrefaction of tissue with attendant bacterial and fungal neoformation of alcohol and other volatile organic substances.[21-23]

Although the timed-interval collection technique for urine specimens cannot, of course, be employed in cadavers; it is of considerable advantage in forensic postmortem alcohol analysis to collect several different body materials in order to be able to estimate the stage of absorption at the time of death. Linck reported urine/blood alcohol ratios varying from 0.74 to 1.70 with a mean of 1.18 in a series of 26 deaths caused by acute alcoholism coming to autopsy[24], and Dubowski in a smaller series of violent deaths with acute alcoholic intoxication found a mean urine/blood alcohol

ratio of 1.18 with extremes from 1.06 to 1.35[25]. Combined postmortem urine and blood alcohol determinations yield considerably more information than either analysis alone. If the urine alcohol level exceeds that of postmortem blood by more than 35%, assuming that the analysis results represent the actual levels of both at the time of death, it is obvious that a blood alcohol level existed at some time prior to death greater than either the urine or blood concentrations found postmortem, and that the subject at the time of death was in the postabsorptive state. Conversely, if the postmortem blood alcohol level equals or exceeds that of the urine, the subject died in the absorptive phase and therefore probably within a few hours of the ingestion time of the alcohol, which can frequently be fixed with some degree of accuracy. The possible import of such conclusions on explaining the cause and circumstances of death makes it mandatory to collect two or more different body materials in death cases. Our routine is to collect adequate postmortem blood and urine specimens whenever obtainable, and to collect brain tissue and cerebrospinal fluid if these are available, as the next choice.

The site of postmortem blood collection is of some importance, since it has been established that alcohol diffusion into the blood in the great vessels and the heart can occur from a large amount of alcohol in the stomach. Because of the comparative ease with which adequate volumes of blood can be obtained, the heart and great vessels (aorta, pulmonary artery and veins, vena cava) are the usual site of collection. Beside the diffusion problem, one must also consider the composition of a postmortem blood specimen in evaluating the alcohol level, in the light of the above discussion of serum/whole blood differences. Our routine is to attempt to estimate the closeness with which a postmortem blood specimen from the heart or major vessels approaches the probable in vivo state at the time of death by performing a hematocrit determination (using the Wintrobe method), and occasionally a hemoglobin determination (using a modified spectrophotometric hemoglobin method[26]) on the supernatent plasma/serum if grossly hemolyzed; and, if indicated, to correct the blood alcohol level found on an aliquot of the same specimen by appropriate factors based upon the proportion of whole blood in the sample. Adequate mixing of heart blood specimens before withdrawal is important; and if it becomes necessary to withdraw heart blood from a body without autopsy, precautions against dilution of the blood with pleural or pericardial fluids should be employed.

Specimen Quantities

In recent years, with the development of alcohol determination methods capable of operating on smaller samples, there has been

a trend toward collection of lesser quantities. The quantity of specimen required for an analysis should not, however, influence a choice of donor site or sample substance. Because of the possible differences between venous and capillary blood and the ease of dilution of the latter by tissue juices, venous blood specimens should be collected even for analysis by micro and ultramicro-methods. There appears to be no valid reason for collecting larger quantities of urine than are desirable for any other body material, i.e., sufficient amounts for the desired number of replicate determinations, and 10-30 ml. should be ample for any analysis scheme; making the timed-interval collection system practical. While most analytical methods for alcohol operate on milligram quantities, directions for collection of tissue specimens have usually called for obtaining 50 to 500 gram of brain or other tissue. As reported by Freimuth et al. there is no significant variation in alcohol concentration in different areas of the brain[17]; therefore a smaller brain specimen, approximately 10-30 grams, should suffice. In all sampling operations, adequate care to obtain a well mixed aliquot of the substance is more important than the preservation of large quantities, and we have encountered no difficulty in supplying adequate duplicate samples or in repeating analyses with our routine collection scheme based upon the collection of 10 grams of tissue or 10 ml. of fluids, whenever that much is available. In general, container size should be proportionate to the sample quantity collected, a one-half ounce French square glass vial with disposable cork lined screw top being found very convenient for fluid specimens.

Precautions

Because of the occasionally occuring claim in the literature that skin sterilization with alcohol may cause an apparent blood alcohol level of up to 0.12%[10], it is good practice to employ a non-volatile skin antiseptic. Aqueous mercuric chloride solution 1:1000 has been used for this purpose; but we have found it convenient to employ routinely a 1:1000 tinted aqueous solution of benzalkonium chloride*, a cationic detergent and germicide, and to cover the puncture site with a 1x3 inch adhesive bandage. With this regimen we have never encountered an infection, nor legal difficulties. Actually, controlled laboratory studies have consistently demonstrated that use of ethyl alcohol for pre-venepuncture skin preparation increases any existing blood alcohol level less than 0.01%[27, 28]. It now being well established that viral hepatitis can be transmitted through imperfectly sterilized needles and syringes, we have routinely employed for venepuncture syringes sterilized by conventional autoclaving followed by exposure to dry heat at 170°C.

*Zephiran Chloride, 1:1000 aqueous solution, Winthrop-Stearns, Inc., New York 18, N. Y.

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for four hours, and sterile disposable needles.* The recently developed paper syringe envelopes**, which can be subjected to the above sterilizing routine, have proven very convenient in our practice. For skin puncture for capillary blood specimens, we employ individual disposable sterile blood lancets#.

II. Preservation of Specimens

Ideally, forensic alcohol determination requires a specimen with ethanol content unchanged since the time of sampling. The nature and extent of preservative measures required to maintain as nearly as possible the original alcohol content of biological substances depend largely upon the expected time interval between procurement and analysis of the specimen, the nature of the sample, and the conditions under which it is to be kept. From both the legal and analytical points of view, it is desirable to refrain as far as possible from adding preservatives or other substances to body material specimens. It has consequently been our practice with all tissue samples to place these immediately after removal from the body individually into new, clean polyethylene freezer bags, seal these, place them into plastic-lined cylindrical cardboard cartons of sufficient size to contain the specimen, and to preserve them solely by storage at −20°C. in an electric freezer cabinet equipped with a temperature recording and alarm system[29, 30]. With this routine, we have found no change from the original alcohol content of brain tissue after sixteen months. Experiments reported by Freimuth et al. demonstrated that tissue storage in closed glass vessels at ordinary refrigerator temperature, 5°C., is not capable of preventing appreciable changes from the original alcohol content, the brain alcohol level decreasing from 0.22% to 0.12% and the liver alcohol level increasing from 0.00% to 0.14% within 14 days[17]. The preservative effect of the lower freezer temperature is most likely due to prevention of alcohol neoformation through putrefactive changes as well as inhibition of the enzymatic oxidation of the original alcohol contents.

The preservation of blood and urine specimens has been the subject of much discussion. In 1945, Kaye and Dammin reported that addition of 7.5 mg. sodium fluoride and 5.0 mg. sodium citrate to each ml. of non-refrigerated blood maintained the original alcohol concentration up to 72 hours[31]. Since our routine practice includes analysis of distantly originating specimens occasionally not delivered to our laboratories within 72 hours after procurement, we evaluated several combination anticoagulants

*Will Ross, Inc., Milwaukee 12, Wis.
**Security Envelope Co., 406 Portland Ave., Minneapolis 15, Minn.; Will Ross, Inc., Milwaukee 12, Wis.
#"Hemolet," Dade Reagents, Inc., Miami 35, Fla.; "Sera-Sharp," Propper Mfg. Co., Inc., Long Island City 1, N. Y.

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and preservatives. For some years, we used 150 mg. sodium fluoride and 100 mg. sodium citrate in containers carrying directions for collection of 10 ml. blood, depositing the salts in film form from 4 ml. of solution by drying in an oven at 150°C. This mixture preserves the original alcohol contents of blood without change for at least 18 days without refrigeration; for well over one month when combined with refrigeration at 5°C., and apparently indefinitely when combined with "deep freeze" storage at −20°C. We have not noted any marked etching of glassware mentioned by Bradford as a disadvantage of fluorides[32], but have occasionally found some small degree of clotting after refrigerated storage of the preserved blood for periods greater than one month. We now use 150 mg. sodium fluoride as a preservative plus 0.5 mg. of heparin sodium as an anticoagulant for up to 10 ml. of blood, and have thus eliminated the clotting completely while retaining the original alcohol concentration to the same extent as with our former fluoride-citrate mixture. Urine specimens are preserved in our laboratories with 15 mg. sodium fluoride in dried film form per ml. urine, combined with refrigeration; benzoic acid has proven equally effective in our hands.

When fluid specimen aliquots are measured by volume for analysis, the effect of the added substances on the original specific gravity of the sample can be neglected for all practical purposes, while such is not the case if the aliquots are to be weighed. For rapidity and accuracy in measuring liquid samples, we routinely employ Ostwald-Folin pattern volumetric pipettes calibrated to contain, which are washed out into the diluting water*.

III. Techniques for the Separation and Isolation of Ethyl Alcohol

Separation Techniques

Techniques for the separation of alcohol from biological material can be classified as follows:

a) Aeration
b) Diffusion-Desiccation
c) Distillation
   (1) Direct-Simple distillation
   (2) Steam distillation
   (3) Reduced pressure distillation

In the form employed in the published alcohol determination methods, aeration, diffusion-desiccation and vacuum distillation are all employed to transfer the alcohol directly from the containing biological medium to an oxidizing solution, usually potassium dichromate in sulfuric acid. All three separation techniques,

*Cat. No. 8201, Arthur H. Thomas Co., Philadelphia 5, Pa.

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therefore, are subject to the same forsensically disqualifying defect—in the event of a positive test, it is impossible to perform any qualitative identity tests for ethanol upon the specimen used for quantitation. The same defect exists in a few distillation methods in which the distillate is delivered directly into an oxidizing solution.

Since all presently described quantitation techniques for ethyl alcohol which are suitable for routine forensic use are to some extent non-specific for ethanol, only direct and steam distillation are suitable methods for the separation of alcohol from matrices, in that they allow qualitative tests upon aliquots of the same distillate used for quantitation. For tissue analysis, steam distillation is generally accepted as necessary; and our routine for tissues includes a preliminary steam distillation using a simple semimicrodistillation apparatus[33]*, the resultant distillate subsequently being treated like other fluid specimens[34]. For non-tissue specimens, steam distillation has no advantage over direct simple distillation; in fact Dubowski found in a controlled study of alcohol determination methods that steam distillation, while reducing the total determination time for a single alcohol analysis from 20 to 15 minutes, also decreased the recovery of added alcohol from 99.7% to 97.1% compared to direct distillation,[35, 42] a phenomenon also noted by other investigators.

In designing alcohol determination methods involving distillation, one should recall that even without any reflux effect 98% of any alcohol present in the sample is found in the distillate volume equal to 1/5 of the total still charge[36]. Since few quantitation techniques have average errors amounting to less than ±2% of the alcohol quantity actually present, there is no recovery advantage to collecting a distillate measuring more than 20% of the still charge volume; and conversely methods such as the author's which includes the collection of a distillate equal to about 40% of the original still charge volume (for other reasons) thereby make virtually certain the complete separation of any alcohol present in the alcohol concentration range existing in the still. Combining an initial steam distillation of tissue with a subsequent direct distillation of the steam distillate, as practiced in the author's method, permits ready inclusion of a suitable acid-and-alkaline double distillation step for increasing specificity when dealing with postmortem materials, where elimination of interference is most important[72].

Choice and Effect of Protein Precipitants

It is difficult to understand why some methods specify that the actual alcohol separation process, e.g., distillation, be carried

*Cat. No. SB-1720X, Scientific Glass Apparatus Co., Inc., Bloomfield, N. J.

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out on a protein-free filtrate of the original biological sample. This requirement introduces additional steps into the analysis, with consequent possibilities for evaporation, measurement errors, and a time delay of at least 10-15 minutes. Proteins of fluid specimens, e.g., blood, cause no difficulty in distillation or other separation processes if suitable protein precipitants are added directly to the sample material in the distillation flask[34,35]. Such protein precipitants need to be measured only approximately; and we have found addition of 5-7 ml. of ⅔ N sulfuric acid and 5-7 ml. 10% sodium tungstate, in the order given, to 2.00 ml. blood specimens contained in 10 ml. of distilled water to be completely satisfactory for distillation, eliminating even the need for routine addition of an antifoam agent when distillation is carried out in our apparatus. When an antifoam agent is desired, as in tissue steam distillations, commercial silicone preparations*, sparingly used, are most satisfactory.

The choice of protein precipitant should be dictated by the rapidity and effectiveness of protein precipitation and ease in distilling without objectionable foaming or carry-over. The incidental pH reaction of the still charge, by itself, has no effect upon either the recovery of actually present alcohol or upon the appearance in the distillate of interfering substances from normal fresh human body materials. Numerous misleading statements appear in the published literature concerning this point. Widmark is quoted by Hinsberg as stating that urine distilled in the presence of *acid* yields "values too high by several hundred per cent."[37] Bamford's text on poisons has perpetuated this error through three editions, stating that "analysis of urine must always be made in the presence of *alkali*, the figure for acid urine being invariably too high. . ." and giving the apparent alcohol due to this cause as up to 0.075%.[38-40] Apparently on the basis of this statement in the Bamford text, Rabinowitch censures several investigators for not recognizing this supposed fact[10]; although Muehlberger later refuted the accuracy of this statement[41]. Dubowski in an investigation of blank values in various body materials yielded by 15 methods, including many which distill alcohol from an acid medium, found no apparent ethanol value greater than 0.016% in fresh human urine from healthy subjects, yielded by any method[35,42]. Fresh urine from healthy normal abstaining human subjects when analyzed for alcohol by the method of Dubowski and Withrow, including acid distillation, consistently yields an apparent ethanol concentration of less than 0.001%[34,42]. To test

*Dow Corning Antifoam A Spray or Dow Corning Antifoam AF Emulsion 30%, (used as a 10% emulsion): Dow Corning Corporation, Midland, Mich.

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further the validity of Bamford's statements, a series of urine specimens containing added known quantities of alcohol were distilled after addition of four different reagent mixtures, including three yielding acid distillates, in very rapid distillation. The results, shown in Table II, definitely prove that the urine need

Table II

Effect of Protein Precipitants on Alcohol Recovery from Urine

Test	Protein Precipitant	Distillate Reaction	EtOH added, %	Recovery, %
1	None (Distilled water)	Neutral	0.100	86.0
2	None (Distilled water)	Neutral	0.150	94.7
3	Picric-tartaric acids	Neutral	0.100	99.5
4	Picric-tartaric acids	Acid	0.150	94.7
5	Sodium hydroxide	Alkaline	0.150	100.0
6	Na tungstate-sulfuric acid	Neutral	0.100	100.0
7	Na tungstate-sulfuric acid	Acid	0.150	94.7
8	Trichloracetic acid	Acid	0.100	95.0
9	Trichloracetic acid	Acid	0.150	102.6

not be alkalinized before distillation to avoid falsely high alcohol concentrations. The data indicate, however, that there is some relation between degree of alcohol recovery and efficiency of the separation process, as characterized by speed of separation, amount of refluxing, and carry-over. Consequently, those methods incorporating the less efficient separation processes, indicated by carry-over resulting in distillate reaction change from neutral, will generally result in lower alcohol recovery regardless of original pH reaction of the still charge.

Precautions and Limitations

If the separation of alcohol is to be followed by its quantitation on the basis of its reducing power on such oxidizing agents as potassium dichromate or potassium permanganate, the quality of the distilled water used for distillation or other dilution is of extreme importance. The distilled water used should be tested at frequent intervals[43], since it deteriorates rapidly in storage, particularly if in contact with rubber tubing and stoppers, and tends to absorb considerable organic reducing substances. Substitution of deionized water for distilled water has been known to occur in some laboratories in the erroneous belief that both were equally suitable for all chemical analyses: deionized water is, however, distinctly non-suitable for forensic alcohol determinations.

Since the first publication of a modified diffusion-desiccation method employing filter paper as a sample container by Abels[44], a number of methods have attempted to obviate the need for special glassware, such as the Conway cell or Widmark flask, or to increase diffusion rates by use of filter paper. Anderson[45] experienced some difficulty with the filter paper diffusion methods of Abels and of Levine and Bodansky[46], and mentioned contamination with

organic matter as a possible source of error. Dubowski investigated the Levine and Bodansky method as typical of some filter paper diffusion methods and found that for alcohol-free distilled water specimens treated like blood samples, apparent alcohol values up to 0.114% could be obtained[42]. The entire procedure was characterized by irregular performance, as was also noted by Hemingway et al.[47]. Our experiments indicate that at elevated temperatures, like those employed in some other recent filter paper diffusion methods[48], actual destructive distillation of the paper occurs and that aberrant alcohol analysis results are not merely caused by oxidation of particles which have fallen into an acid dichromate mixture. One recent method, that of Smith[49], has successfully avoided such difficulties, in its author's hands, by employing less severe oxidation conditions, 15 Normal sulfuric acid and a maximum temperature of 60° C.

The objection to methods other than distillation for complete forensic alcohol determinations because of their inability to yield aliquots of the quantitated specimen for qualitative alcohol tests is, however, no bar to their employment for screening tests. In forensic laboratories which handle large numbers of fresh fluid specimens of unknown alcohol content, such as coroner's or medical examiner's offices, the various diffusion-desiccation methods based upon the use of Conway cells or modifications thereof[50-53] are ideal for rapid screening with micro samples and minimum analytical attention; and we employ at times a simple modification of the Dubowski and Withrow method[34], using 0.20 ml. blood specimens and a 68 mm. Pyrex glass Conway cell* for rapid and simple screening of totally unknown postmortem samples. With specimens from living subjects with relatively good histories, as in routine examination of drivers for alcoholic influence, the screening should not be required and distillation methods appear preferable.

IV. Techniques for the Identification of Ethyl Alcohol

In forensic alcohol determinations, one must be able affirmatively to establish that any positive findings were wholly attributable to ethyl alcohol, if reported as such, or to such other substances as were present and reported. In practice, therefore, in laboratories reporting analysis results in terms of ethyl alcohol, the use of either a method completely specific for ethanol or the use of a method as selective as practicable for ethanol combined with qualitative tests proving the presence of ethanol and the absence, beyond a reasonable doubt, of other interfering reducing substances is mandatory. The former type of analysis is at present not wholly satisfactory for routine forensic use.[54-58] If distillation is

*Cat. No. J-2162, Scientific Glass Apparatus Co., Inc., Bloomfield, N. J.

used to separate the volatile reducing substance from the containing material, ample material is usually available for qualitative tests. Our routine employs a comparatively selective quantitation method affected only by the aliphatic alcohols, paraldehyde and formaldehyde, but not by acetone in any blood, urine or cerebrospinal fluid concentrations found in living subjects. Therefore, with antemortem materials, we routinely test the distillate for presence of ethanol; for absence of methyl alcohol and formaldehyde with our modification of Ozburn's chromotropic acid test[59], and for absence of other aldehydes with Schiff's reduced fuchsin test[60]. As an extra precaution, we routinely test a drop or two of the distillate for acetone, before oxidation, and for isopropyl alcohol (by repeating the acetone test after oxidation)[61], with a commercial urine acetone test tablet*, which is sensitive to acetone concentrations of 0.01%, well below any interfering acetone level for our method. Paraldehyde in interfering concentrations can also, of course, be detected organoleptically in the specimen, as can formaldehyde.

In alcohol determinations performed upon drivers or pedestrians, volatile reducing substances other than ethyl alcohol and, occasionally, acetone are rarely encountered. Occasionally, a subject is found as a driver or more often as a pedestrian suspected of being under the influence of alcohol, who has become markedly disabled through the combined effects of ethyl alcohol and paraldehyde. We have encountered a series of such persons, known chronic alcoholics; but in no case had any difficulty in detecting by odor the presence of paraldehyde in the blood specimen submitted, even when present only in minimally interfering concentrations. The extent of possible errors due to presence of paraldehyde in alcohol determinations employing the potassium dichromate-sulfuric acid system for quantitation is indicated by the examples of blood analyses from pedestrians involved in vehicular accidents given in Table III[62].

Very rarely, a living subject is encountered who has ingested mixtures of methyl and ethyl alcohol ("smoke"). The qualitative tests mentioned above will reveal the presence of as little as 2 micrograms methyl alcohol per ml. distillate and this can be confirmed and quantitated by the techniques outlined by Gettler[63], Boos[64], Ozburn[59] and others. In two instances in our experience, subjects were found in parked motor vehicles unconscious following ingestion of a mixture of ethanol, methanol, and acetone. The qualitative tests outlined demonstrated presence of each of these substances in the blood.

*ACETEST Reagent Tablets, No. 2381, Ames Co., Inc., Elkhart, Ind.

Table III
Effect of Presence of Paraldehyde on Ethanol Determinations.

Case	Paraldehyde Level, %	Apparent Ethanol Level, %	Actual Ethanol Level, %
1	0.067	0.57	0.48
2	0.034	0.09	0.08

A serious source of interference in forensic alcohol determinations may be the use of contaminated containers for blood or urine specimens; though this is most common in fatal cases where blood or other specimens have been collected in embalming fluid, morturary antiseptic, or similar containers. The possibility of such contamination should be obvious from inspection and is simple to avoid by generous distribution of properly prepared containers to all potential users, with subsequent prompt automatic replacement of used units.

The exact nature and concentration of interfering substances to be guarded against will depend upon the particular alcohol method employed and the type of specimens to be analyzed. A partial survey of false apparent alcohol values yielded by 10 methods has been reported by Dubowski[42]. In forensic practice, as stated above, the most practical approach is to employ a method relatively selective for ethanol, and to test for possibly interfering concentrations of the more common interfering substances by qualitative tests. For analysis on fresh material from living subjects these precautions are adequate, when combined with a qualitative test enabling the analyst to state affirmatively that ethyl alcohol was actually present in the specimen analyzed. Each forensic laboratory should take the precaution of analyzing a series of body material specimens containing various added concentrations of possible interfering substances, in order to establish the response of the method employed and to be prepared for possible court examination citing such reports as that of Möllerström who reported, using a modified Friedemann and Klaas method, finding apparent alcohol levels up to 0.49% in presumably abstaining diabetic subjects[65].

Occasionally, it is desirable for scientific or legal reasons to perform forensic alcohol determinations in duplicate. Where this routine is employed, qualitative identification of any ethanol present can be conveniently combined with quantitation simply by employing two or more chemically different quantitation procedures (e.g., acid dichromate oxidation and acid permanganate oxidation) upon aliquots of the same sample or sample distillate[66-68]. Numerous simple variations of this principle are possible, such as oxidizing a single distillate under equal time and temperature conditions with two potassium dichromate solutions varying in

sulfuric acid concentration, or changing oxidation time, oxidation temperature or other variables in replicate analyses with a single reagent. If the only reducing substance present is ethanol, analytical results calculated as ethanol under these varying conditions or yielded by different reaction systems will coincide within the combined respective analytical precision limits, and thus identify the unknown as ethyl alcohol. If the analytical results, expressed as ethanol, show marked discrepancies beyond the expected precision limits, part or all of the reducing substance present is not ethanol.

V. Techniques for the Quantitation of Ethyl Alcohol

The number of methods applicable to quantitation of ethanol separated from human body materials is too great to permit extended consideration here, but all of the many schemes proposed employ one of the following 9 principles:

a) Oxidation of ethanol to acetic acid and titration of the latter
b) Oxidation of ethanol with standard potassium dichromate in acid solution to a green endpoint
c) Oxidation of ethanol to acetaldehyde and quantitation of the latter
d) Formation and estimation of iodine derivatives of ethanol, e. g., iodoform
e) Quantitation by means of physical properties (e.g., interferometry, mass spectrometry, refractive index measurement, polarography, etc.)
f) Conversion of ethanol to ethyl nitrite and measurement of its hydrolysis followed by release of free iodine from potassium iodide
g) Oxidation of ethanol to acetic acid with known excess oxidizing agent and calculation of the ethanol oxidized from the quantity of oxidizing agent reduced, as determined from the quantity of reaction products resulting or by measurement of the excess oxidizing agent remaining by titration, photometry, spectrophotometry, visual color comparison, etc.
h) Enzymatic dehydrogenation of ethanol with alcohol dehydrogenase with simultaneous reduction of diphosphopyridin-nucleotide to dihydro DPN, and measurement of the latter spectrophotometrically
i) Pyrogenic conversion of ethanol to ethylene followed by bromination, liberation of free iodine and measurement of the latter.

We have found that the reduction of potassium dichromate in sulfuric acid solution under precisely controlled conditions is nearly ideal for routine forensic use. The reaction between ethanol

and potassium dichromate in acid solution is well known and understood, and has been found by many observers, including Dubowski[35], to be stoichimetric and readily controllable. We currently determine excess dichromate photometrically; and it seems likely that some of the newer spectrophotometric techniques for improving the sensitivity or ease of dichromate ion measurement, such as its measurement as the s-diphenylcarbazide complex at 540 millimicrons as suggested by Williams and Reese[69], or the displacement spectrophotometry technique can profitably be adapted to this problem.

Precautions for the Acid-Dichromate Method

When potassium dichromate in acid solution is used to quantitate ethanol, a few precautions are required. The quality of the sulfuric acid with respect to freedom from organic reducing substances is important, and this latter property as well as the freedom of the distilled water from organic contamination can be determined easily by the permanganate reduction test as follows:

> Three volumes of concentrated sulfuric acid are added to 4 volumes of distilled water, using chemically clean apparatus and precautions to avoid contamination. To 10 ml. of the cooled mixture is added 1 drop of N/20 potassium permanganate, and the resulting pink color observed. If both the sulfuric acid and the distilled water are sufficiently free from organic reducing substances, the pink color remains for at least 15 minutes.

The acid dichromate solution is somewhat light sensitive and should be protected from excessive light. Experiments in our laboratories have also demonstrated that the newer polyethylene reagent bottles, while possessing many desirable features, reduce to an appreciable degree acid dichromate solutions, and these must therefore not be stored in such plastic containers.

Technique

When potassium dichromate in sulfuric acid solution is employed to quantitate ethanol solutions, it can be relied upon as a primary standard if prepared with the usual precautions and strictly quantitative technique. In photometric determination methods yielding final results from calibration tables or curves, precision depends in great part upon the complete reproducibility of all quantitative steps of the method. For this reason and for rapidity and ease of routine analyses, we have found it convenient to deliver the acid dichromate solution, the only volumetrically critical reagent in our methods[34, 70], from a light-protected all Pyrex automatic pipette*. These can be supplied in non-standard volumes on special order. Similarly, in order to obtain reproducibility in the heating of the acid dichromate and distillate mixture, we prefer to use an electric constant temperature bath of adequate

*Cat. No. 71-645, E. Machlett & Son, 220 East 23rd Street, New York 10, N. Y.

depth, with an inhibited polyalkylene glycol as the bath fluid#.

There appears to be some confusion in the literature on the exact quantity of potassium dichromate equivalent to 1 milligram of ethanol. From the stoichimetric relations of the reaction

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

it follows that 4.2576 milligrams of potassium dichromate are equivalent to 1.0000 milligram of ethanol.

VI. Postmortem Alcohol Determinations

Occasionally, postmortem alcohol determinations become necessary as a result of traffic deaths. The feature distinguishing such determinations from other postmortem alcohol analyses is that, generally, the subjects are examined shortly after death and that autopsy specimens received for alcohol determination are, therefore, usually not appreciably decomposed.

The concentration of alcohol found in postmortem body fluids or tissues is a function of the following factors:

(1) Concentration of alcohol actually present originally
(2) Percent recovery of actually present alcohol, a characteristic of the analytical method
(3) Postmortem loss of alcohol originally present before and/or after removal of specimens from the body due to such causes as diffusion into neighboring structures, enzymatic oxidation, evaporation, etc.
(4) Postmortem gain of alcohol as a result of neoformation of alcohol before and/or after removal of specimens due to enzymatic fermentation or other factors, diffusion from adjacent tissues, etc.

In addition, there is the problem of apparent alcohol concentrations yielded by the analytical method employed in the presence of various concentrations of volatile reducing bodies produced by postmortem decomposition and putrefaction, such as aldehydes, amines, ketones, sulfides, phenols, etc. It is apparent that during prolonged intervals between death and the removal of tissues or fluids for alcohol determination, the various factors can operate jointly to reduce or obliterate any original alcohol level, to reproduce accidentally by loss and neoformation the original concentration, or to produce a considerable apparent or true alcohol concentration in a body free of alcohol at the time of death. Except in putrefied water submersion cases, loss of pre-existing alcohol during putrefaction generally exceeds alcohol gain from neoformation[71]. The latter tends to reach the range of borderline intoxication as a limit, although rare neoformed alcohol levels of, up to 0.42% have been reported in advanced putrefaction[17]. There-

#UCON Fluid 50-HB-280-X, Carbide & Carbon Chemicals Co., 30 East 42nd St., New York 17, N. Y.

fore, high positive ethanol findings in cases having undergone putrefaction under conditions other than water submersion ordinarily furnish evidence of the existence of equal or higher alcohol concentrations at the time of death. Alcohol neoformation in markedly putrefied submersion cadavers ("floaters") generally exceeds that in non-submersion cases of comparable decomposition, probably as a result of intensified anaerobic bacterial fermentation processes[71], and alcohol levels from such material must be evaluated cautiously.

In the typical vehicular accident death, autopsy is performed before any appreciable decomposition has occurred, and under such conditions precautions to insure finding of the original alcohol level need not be too elaborate. When postmortem materials obtained within a few hours after death are submitted to our laboratories promptly, we have found the unmodified Dubowski and Withrow method[34] to yield accurate results. When a somewhat longer interval between death and submission of sample material has occurred, the Dubowski and Withrow procedure is combined with a double distillation of tissue steam distillate or fluid samples, using the reagents of Kozelka and Hine[72]. The final distillate is tested for aldehydes and ketones; and redistilled, if necessary, from both acid and alkaline media. Our usual acid potassium dichromate quantitation then follows. In common with other investigators, we have found that this double distillation results in postmortem blood and brain alcohol levels 0.020 to 0.038% lower than the unmodified Dubowski and Withrow method when applied to partially decomposed specimens.

As pointed out under the discussion of sampling criteria, no attempt should be made to estimate the antemortem or death concentrations of alcohol in one organ or fluid from that found postmortem by analysis of another. The existing alcohol concentration ratios, however, are of obvious significance in the evaluation of the absorption stage at time of death and the estimation of time of death on the basis of known alcohol ingestion times[24], and therefore alcohol analyses on blood, brain, and urine should be performed in fatalities whenever these materials are available. A few of our cases, summarized in Table IV, illustrate the fallacy of the attempt to calculate probable blood alcohol levels at time of death from postmortem urine alcohol determinations using the commonly accepted urine/blood alcohol ratio of 1.30.

It is apparent that one application of a postmortem urine alcohol determination in conjunction with the blood alcohol level is to establish a "least maximum level" which the blood alcohol must have reached at some time prior to death. Thus in Case 1 of Table IV, it can be seen that although the actual blood alcohol level found was 0.19%, from the urine alcohol concentration the

Table IV
Correlation of Postmortem Blood and Urine Alcohol Levels

Case	Urine Alcohol Level, % W/V	Blood Alcohol Level, % W/V	Urine Alcohol / Blood Alcohol	Calculated Blood Alcohol Level, % W/V
1	0.26	0.19	1.35	0.20
2	0.28	0.24	1.15	0.21
3	0.30	0.28	1.06	0.23
4	0.38	0.33	1.16	0.29
			Mean 1.18	

blood alcohol level prior to death must have been at least 0.20% at one time; and was in fact somewhat higher since the urine represents a pooled sample at least some of which was excreted after the maximum blood alcohol level had been surpassed.

VII. Analytical Interpretation of Test Results. Unusual, Unexpected and Bizarre Results.

Analytical Interpretation of Test Results

A necessary corollary to valid physiologic interpretation of alcohol test results in terms of possible alcoholic influence is the correct interpretation of the analysis results in terms of actually existing alcohol concentrations at a given time. In addition to the matters discussed above, interpretation of the results of an alcohol analysis is subject to certain purely technical and scientific considerations not related to any physiological aspects.

The statutory definitions of alcoholic influence or intoxication promulgated in the United States, and expert testimony concerning the same in other jurisdictions not having statute law on the matter, uniformly express blood and other alcohol levels in terms of weight/weight per cent. It is, however, common practice in this country to perform practically all clinical chemical determinations upon volumetrically measured specimen aliquots. Such volumetric measurement is desirable for certain technical reasons, such as the change from original density caused by preservatives, and for the expediency of greater speed and sufficient precision. If, however, in interpreting the reported tissue and fluid alcohol levels the deviation from weight/weight percentage is not known or neglected, significant errors can occur. Blood, for example, has an average specific gravity of 1.055 and may have an even greater density if the hematocrit value is unusually high. Thus a blood alcohol level of 0.155 weight/volume %, i.e., 1.55 mg. per ml., is equal to a level of only 0.147 weight/weight %, i.e., 1.47 mg. per gram. When these values are reported to

two decimal places and without specification, they become, respectively, "0.15%" and "0.14%." A hairline legal interpretation made without awareness of the difference caused by the volumetric sampling could conceivably result in a decision different from that which would have resulted from a report on the weight basis, even though the difference is physiologically negligible. Obviously, consequential legal decisions should not be based upon such narrow limits; although the jurist does require some set borderline limit for consistency, like the vehicular speed limit in another aspect of traffic law enforcement. The unspecified alcohol level report based upon a weight/volume sample relation, however, in part defeats the original intent of the legal definitions imbodied in the interpretation statutes. The remedy lies in factual reporting of blood and other alcohol concentrations in terms of the actual measurement involved, accompanied by estimated recalculation to any other system of measurement of interest; or, better, by an explanation of the deviation limits attributable to the difference in measuring technique. We habitually report all fluid alcohol determination results in terms of per cent W/V and in our interpretation take this factor into account.

Forensic alcohol determination results should be expressed in a factually correct and physiologically meaningful manner. In general, all quantitative analytical results should include a statement of the precision limits attached to the measurement. This may be done by reporting a given alcohol level to the first doubtful figure and adding the precision limits (e.g., 0.193 ± 0.004 % W/V) or by reporting only two significant figures (e.g., 0.19 % W/V). The latter report is equally meaningful physiologically and legally, and represents sounder forensic practice. In expressing the results quantitatively at all, the analyst implies that the report refers to a single substance determined, universally understood to be ethyl alcohol. The validity of such implication depends upon the specificity of the particular method employed for determining alcohol. It is, consequently, better analytical practice and certainly sounder legally to establish experimentally to a reasonable degree of certainty the presence of ethanol and the absence of any substances which could react like ethanol in the analytical system used, than to rely on assumptions that no such other substances were present in the specimen.

A related matter of interpretation not under the control of the person making a physiological interpretation of an analysis result is the concordance between a reported alcohol level representing true ethanol and the original alcohol level in the sample material at the time the sample was obtained. It is incumbent upon the analyst to make every reasonable effort not only to preserve the

specimens in as nearly as possible the original state in which received by him until the analysis, but also to indicate in the report any likelihood of a significant difference between any alcohol concentration found and the probable original concentration. By this it is not intended to urge the analyst to estimate the degree and direction of any suspected deviation, but rather to indicate the fact of the actual or suspected existence of such deviations, so that the weight attached to the report for legal purposes can be commensurate with this factor.

Unusual, Unexpected, and Bizarre Results

Such results require special consideration. In traffic alcohol determination practice, the great majority of blood alcohol levels found lie between 0 and 0.30% W/V, with an occasional higher value in vehicular deaths or other unusual circumstance. The availability of a history or physical evaluation of the subject in most traffic alcohol determination situations offers the opportunity to check the analytical results against expectable ranges. Any grossly unusual result should, of course, be rechecked with scrupulous care to avoid introduction of any systematic or personal errors in the analysis; and in smaller laboratories handling only occasional alcohol determinations, a check analysis on a known concentration standard should always be performed simultaneously with the unknown. This is a worthwhile procedure for large laboratories as well.

Due allowance must be made for physiological variations in evaluating the probable correctness of analytical results. Unexpectedly low values, upon confirmation, often are found to have their basis in discrepancies in time between the collection of the specimen and the observation period reported. It is good practice to make available to the person evaluating the analytical result for accuracy some such detailed record as the National Safety Council ALCOHOLIC INFLUENCE REPORT FORM. The laws of several states provide that any alcohol determinations undertaken to establish presence or absence of alcoholic influence at the time of an alleged motor vehicle offense must be performed on specimens obtained within two hours of that time; and lesser intervals are very desirable. Unusually low alcohol levels of the blood, in the face of reported coma or marked disorientation of the subject may arise from several common causes. Not infrequently, motor vehicle operators, passengers, or pedestrians are found to be under the simultaneous influence of both alcohol and other substances, most commonly the several narcotics, hypnotics, and sedatives; and somewhat more rarely, carbon monoxide, Disulfuram, or other drugs. The synergistic effects of alcohol and the barbiturates are well known and are frequently observed in forensic practice. Less well known is the at least equally potent syner-

gism between alcohol and paraldehyde, seen occasionally in pedestrians who become involved in motor vehicle accidents. In many thousands of alcohol determinations, we have observed only two instances of apparently bizarre blood alcohol levels explainable on the basis of diabetic acidosis, and two instances of marked disorientation traceable to ingestion of alcohol combined with smoking of marihuana. Complete toxicological and clinical chemistry facilities are, of course, very desirable in the further investigation of unexpected or bizarre alcohol levels; and the analyst should not hesitate, after immediate recheck and reconfirmation of the analytical result, to advise hospitalization or other further medical attention of a subject yielding such analytical result. Motor vehicle accidents involving violence not infrequently lead to head injuries or other serious medical complications and such possibilities should be kept in mind. Under some conditions (prolonged parking with the automobile engine running to heat the cars, waiting in line during traffic jams with car ventilators open and fans operating, etc.) it is possible for drivers and passengers to inhale sufficient carbon monoxide to become markedly affected, and this possibility should also be considered when appropriate.

A request to police authorities for a search or re-search of a subject's person, clothing, or automobile on the basis of unexpected analytical alcohol test results may occasionally produce evidence of the possession and use of narcotics or other drugs, or one of the several types of warning cards identifying the subject as a user of "ANTABUSE", or as a diabetic, overlooked in the original search.

It is obvious that all material in the possession of an analyst relating to one of these bizarre forensic alcohol levels should be preserved with due regard for its evidentiary value for any desired later procedures; and where indicated and possible, it is good practice to obtain as soon as the unexpected result becomes known duplicate specimens of the same and another body material for re-checks.

Unexpectedly high alcohol levels are occasionally found in motor vehicle operators or passengers, and somewhat more often in pedestrians. We have encountered numerous rechecked blood alcohol levels exceeding 0.25% W/V in drivers, and have also found a series of blood alcohol levels between 0.30 and 0.35% W/V in operators able to walk. Our highest blood alcohol level found in a conscious pedestrian was 0.43% W/V with simultaneous pooled urine alcohol concentration of 0.32% W/V, both obtained within 15 minutes of an automobile accident in which this subject was involved. There has been a pronounced scarcity of recent publication of the results of large series of body ma

terial alcohol levels found in some active forensic alcohol determination program; and it should be of great interest and value for some of the more active law enforcement laboratories to make available their data on this subject.

Conclusion

In the light of the scientific and legal implications of ethyl alcohol determinations, particularly when these are accepted without critical evaluation, it is apparent that only institution and supervision of forensic alcohol determination programs and systems by those with adequate technical and forensic experience can avoid the many pitfalls inherent in the technology and application of such tests. Such guidance is available through the National Safety Council's Committee on Tests for Intoxication*.

It is interesting to note the commendable trend toward more rigid legal regulation by statute and case law of conditions under which the results of forensic alcohol determinations may be accepted in evidence. Thus, in the State of Nebraska, analysis of body fluids for alcoholic contents may be performed only by persons approved by the State Department of Health, employing the specific methods stated in the application for such approval; the purpose of the statutory regulation being to assure the competence of test results by requiring departmental approval of individuals making the tests[73]. Recently, a case decision of the Court of Criminal Appeals of Texas held the results of a chemical test for alcohol (in the breath) inadmissable because proof was lacking that the operator and the equipment employed were under the periodic supervision of one who has an understanding of the scientific theory of the alcohol determination method employed[74, 75]. With the ever-increasing use of forensic alcohol determinations and the correspondingly more acute shortage of forensically qualified and experienced analysts only sound analytical practice coupled with legislative or judicial control insuring truly expert supervision over forensic alcohol determinations can properly safeguard the interests of both the public and the individuals subjected to such tests.

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