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GUIDE TO URINE TESTING IN DRUG ABUSE PREVENTION AND MULTIMODALITY TREATMENT PROGRAMS

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1. INTRODUCTION

The usage of drugs both legal and illicit is still on the increase and appears to be a continuing phenomenon. However, the pattern of drug use varies from time to time depending on the availability of an abused drug in the illicit market. A recent shift from narcotics to poly-drugs use has caused much concern among the drug abuse prevention programs and Law Enforcement Agencies. In order to attack the problem of drug abuse more realistically, it is imperative that we distinguish between the different drugs which are abused. The effort to control the abuse of different drugs has generated an urgent demand upon clinical chemists and toxicologists to utilize their collective expertise in developing not only new, specific, sensitive, versatile but also simple and inexpensive qualitative techniques for the detection of new types of abused drugs. During the past eight years the number of out-patient and in-patient multimodality treatment programs has been increased. They have been established in the U.S.A. by federal agencies such as Special Action Office for Drug Abuse Prevention (SAODAP) and the National Institute of Alcohol, Drug Abuse and Mental Health; state and local agencies; and private organizations including community groups. The overall objectives of this report are to provide to the non-toxicologist the following information pertaining to urine testing: (i) the purpose of urine testing, (ii) basic knowledge about the dynamic nature of abused drugs in the body for correct inter-

pretation of urinalysis data, (iii) definitions of commonly used terms in urine analysis, (iv) comprehensive review of existing detection procedures pertaining to drugs of abuse and drugs used in the treatment, (v) setting up of toxicology laboratory facilities and cost of analysis for testing more than one drug per urine specimen.

The author sincerely hopes that physicians, clinicians, therapists, program directors and drug counselors who are involved in the day-to-day management and counseling of drug-dependent individuals in multimodality treatment clinics will be able to make better clinical interpretations of urinalysis data in more effectively combating the drug abuse problem. Additionally, this guide should enable drug administrators and/or executive and clinical directors to make meaningful decisions in the choice of an appropriate toxicology laboratory facility, the types of drugs to be tested by urinalysis, the treatment monitoring efficacy and the continuing evaluation of the need for additional treatment. A better visibility regarding the epidemiological potential of the illicit drugs sold "on-the-street" should result also, it is hoped.

2. WHY URINE SCREENING IS NECESSARY FOR CLIENTS ATTENDING MULTIMODALITY DRUG ABUSE PREVENTION TREATMENT PROGRAMS

Urine screening for determining illicit drug use has become a necessary adjunct to treatment for heroin addiction. Although saliva has been a vehicle for the excretion of certain drugs, *e.g.*, alcohol and morphine[1], the chemical analysis of urine remains the predominant tool for detecting drugs ingested by individuals in drug abuse screening programs. Furthermore, urine is the most convenient source of material for the detection of drugs, can be obtained in adequate quantities without discomfort, and, repeated testing, if needed, can also be performed.

A decision to admit a client to a treatment program should not be based on the analysis of single urine test. Instead, a profile of several urine tests should furnish the particular drug abuse pattern information prerequisite to the choice of treatment alternative. An individual has to use heroin for several weeks or months before developing physical dependence. A proper diagnosis should involve client's history, physical examination and the evaluation of results of comprehensive urine testing, including a wide array of potentially abused drugs.

After the client has been admitted to a suitable treatment modality depending on the clinical needs of the individual, the urine analysis will provide the physician with his only objective measure of progress in treatment and serve as an extremely useful tool for confrontation with the client on his day-to-day clinical management. According to Senay and Renault[2], urinalysis discourages the patient from attempting to fool himself and his counselors about his real behavior. It creates an honest basis for relationship between the client and individuals treating him, and serves as a solid indicator of client's progress in a treatment. In addition, frequent urine testing has a strong deterrent effect on drug users. Kurland and co-workers[3–7] demonstrated the importance of the deterrent effect which the daily analysis of urines produced in a population of previous narcotic users. However, the special requirements of a clinical program for drug detection will depend on the particular clinical goals of a management technique, the resources available and administrative considerations. For example, programs which use drug monitoring procedures as a deterrent to drug use could be far more concerned with accuracy and elimination of false positives than programs

which use results primarily to follow the clinical progress of voluntary patients or to evaluate different approaches. Programs dealing primarily with heroin users who do not commonly abuse other drugs would be less concerned about detection of barbiturates and amphetamines. However, treatment programs where a large proportion of clients abuse amphetamines, barbiturates, tranquilizers and perhaps utilize prescribed drugs for certain illnesses require wide-ranging, sensitive, accurate and low-cost detection procedures. The detection systems used should maximize the differentiation between prescribed medications and their metabolites from illicit drugs and their metabolites and adulterants. The number of urine specimens collected from each patient each week and the nature of the investigational drugs used to treat the patients can also influence the decision of the investigator about the choice of the detection technique. In rare situtations such as Emergency Toxicological Services, economy and other considerations may have to be sacrificed for rapidity of analysis. In programs with long-range treatment approaches where time is not a critical factor and where a detection of a wide variety of drugs of abuse is desired, low-cost, versatile detection techniques should be used.

The author strongly feels that drug-dependent individuals voluntarily participating in treatment programs such as methadone or L-α-acetylmethadol (LAAM) maintenance; narcotic antagonists (naltrexone, naloxone and cyclazocine); transitional care; detoxification and total abstinence; therapeutic communities; Daytop or drug-free houses should be monitored for their drug use activity at a frequency level suggested in our earlier publication[8].

Administering methadone or other drugs of treatment, providing group therapy, legal counseling, vocational rehabilitation, social services, and holding so-called rap sessions between the client and counselors without monitoring the drug activity of the individual essentially precludes comparative measurement of success and, instead, provides fertile ground for endless debate and programmatic double talk. The author does not foresee any alternate method of measuring progress which has any scientific, objective and clinical value other than drug testing. The frequency of urine testing should depend on the clinical progress of each individual in following the program goals. Whereas a client early in the therapy should drop 3 urines or more a week for the first six months, the frequency may be reduced after that time to only 2 drops a week and so on. If a client's urine reports have been consistently "clean" for a period of six months, only then one collection of urine per week on random basis as proposed under current U.S. Federal Regulations would be sufficient as a check for covert drug use.

The current Federal Drug Administration (FDA) Regulations mandating (weekly) testing for morphine and (monthly) testing for methadone, amphetamines, and barbiturates are much too lenient and inadequate. They do not provide a continuity of vigilance of drug using activities of the individuals attending various treatment programs. Certain individuals require multiple urine testing for narcotics and/or poly-drugs every week. Furthermore, many poly-drug abuse treatment programs are being established without making any provision for weekly poly-drug testing. The existing Federal Regulations of (monthly) testing for poly-drugs are vague and include such phrases "once a month test for methadone, barbiturates, amphetamines and other drugs if needed" and have no redeeming value. Unfortunately most of the drug programs and service laboratories interpret the word "Amphetamines" to mean only

one amphetamine (dexedrine, benzedrine). In fact, most of the service laboratories did not have an adequate test for methamphetamine until the U.S. Center of Disease Control at Atlanta included methamphetamine in their list of drugs under "Drug Abuse Toxicology Proficiency Testing".

Similarily, other central nervous system stimulating drugs having high abuse potential such as phenmetrazine (Preludin) and methylphenidate (Ritalin) are neither required for analysis nor does the typical service laboratory have an adequate test available. Federal Regulations under (monthly) testing of poly-drugs should specifically itemize the names of drugs which are alleged to be widely abused, should plan for the required testing of the itemized drugs and should notify all of the schedule for implementing the plan. FDA should revise this list periodically as found desirable with shifts in abuse pattern. Without question, uniform provision for frequent urine testing is imperative if we sincerely desire to help drug dependent individuals.

3. DRUG DYNAMICS AND THE INTERPRETATION OF URINALYSIS DATA

A practical understanding of drug dynamics is a necessary prerequisite for the proper interpretation of urinalysis data. There are different routes of drug administration: oral, inhalation and injection (subcutaneous, intramuscular, intravenous). Drugs taken orally can be retained there and absorbed through oral mucosa or when swallowed are absorbed from the stomach and intestine. Drugs when inhaled (snorted) are absorbed through the pulmonary endothelium or mucous membranes of the respiratory tract and thus gain rapid access to the circulation. Absorption of drugs by injection especially by intravenous route is more rapid and therefore is preferred by drug addicts to achieve immediate desired results. After the drug is absorbed into the blood stream it passess through various body fluid compartments and two processes begin simultaneously: detoxification and excretion.

Detoxification is the metabolism or biotransformation of foreign organic compounds in the body. Biotransformation of drugs occurs mainly in the liver but some also takes place in the intestine and kidneys. The chemical reactions involved in the biotransformation of drugs and other foreign organic compounds can be classified as non-synthetic and synthetic. The non-synthetic reactions involve oxidation, reduction, or hydrolysis and may result in activation or inactivation of the parent drug by being metabolized into one or more end products (metabolites). The synthetic reactions also called conjugations involve coupling between the drug or its metabolite and an endogenous substrate such as sulfuric acid (ethereal sulfate), glucuronic acid, an amino acid, or derivatives of these, *e.g.*, glycine, glutamine, cysteine, ornithine. Synthetic reactions almost invariably result in the inactivation of the parent drug.

Excretion is the process of removing or eliminating the drug or its biotransformed products (metabolites) from the body. The routes of excretion are the kidney (urine), the gastrointestinal tract (stools), lungs, skin (sweat) and mouth (saliva). Drugs excreted in the faeces (stools) are derived either from unabsorbed orally ingested drugs or from metabolites excreted in the bile and not reabsorbed from the intestinal tract. Excretion of drugs in milk is important not because of the amounts eliminated but, due to the fact that the excreted drugs such as morphine (metabolite of heroin) in the case of drug addict mothers and methadone (a treatment drug) could be potential sources of unwanted pharmacological effects to the nursing infant.

Pulmonary excretion (lungs) is of importance mainly for the elimination of anesthetic gases and vapors. The kidney is the principal means of excretion of drugs, therefore, the various tests have been designed to detect unchanged drugs and/or their metabolites in the urine. Drugs can also be detected in other body fluids such as gastric contents, saliva, blood, and perspiration (sweat), but these sources are unsatisfactory for screening large groups of people, particularly the drug-dependent individuals attending multimodality treatment programs. However, efforts are being made to test drugs such as morphine (a metabolite of heroin) in saliva as a mass screening procedure.

Excretion of unchanged drugs and/or their metabolites also depend on a variety of parameters characteristic of drug users such as drug dosage, frequency of drug use, nutritional status, pathological state, amount of exercise, age, genetic and strain differences, body temperature, body weight, daily volume of liquid consumption and concomitant use of other drugs. The physio-chemical properties of the drug itself and influence of pH are equally important parameters. The excretion of amphetamines is markedly affected by the pH of the urine. Excretion is slow in alkaline urine and rapid in acidic urine. The readers interested in further details on biotransformation and metabolic changes of drugs are advised to refer to books on pharmacology and medicinal chemistry[9,10].

The dynamic nature of widely abused drugs and drugs used in the treatment is described in the following sections.

A. Heroin

Heroin is diacetyl morphine, also known as diamorphine. The name heroin is derived from the German word "Heroisch" which means large or powerful. It is prepared from morphine by the acetylation of both the phenolic and the alcoholic OH groups. Heroin is a highly effective narcotic analgesic, pharmacologically similar in action to morphine, although as a pain killer its milligram potency is three to four times greater than morphine[11]. Since it induces vicious addiction, it is no longer prescribed. Heroin is sold under various names such as H., Horse, Scat, Junk, Smack, Scag, Stuff and Harry. Before the heroin reaches its user, it is "cut" by the addition of adulterants such as milk sugar (lactose), quinine, mannitol, corn starch or almost any white powdery substance. The addict usually dissolves the material in boiling water and injects it intravenously which is called "mainlining"[12]. Some beginners inject it under the surface of skin, called "skin popping". Heroin can also be "snorted" or "sniffed" by drawing the powder into the nostrils with a sharp, quick sniff. Habitual sniffing leads to perforation of the nasal septum.

Heroin, in the body is rapidly metabolized by the liver, kidney and tissues to monoacetyl morphine (MAM) which is further biotransformed to morphine. Thus a urine specimen for heroin abuse is always tested for the presence or absence of morphine. Heroin also appears as morphine in breast milk, perspiration and saliva. The following recoveries of heroin and its metabolites in the urine collected for 40.5 h after the intravenous (i.v.) infusion of 10 mg of heroin have been reported (the excretion reached near maximum rates between 2.6 and 4.6 h): total morphine 37.4–46.4% (mean 43%), conjugated morphine 34.3–42.6% (mean 38.3% or 89% of total morphine), free morphine 3.1–5.3% (mean 4.2% or 9.75% of total morphine), 6-

monoacetylmorphine 0.48–2.82% (mean 1.3%), free heroin 0.05–0.3% (mean 0.13%)[13]. Fujimoto and Way[14] also reported that as much as 83% of total morphine may be excreted as its conjugates. It has been demonstrated that morphine 3-glucuronide is a major metabolite.

Because the percentage of heroin in street samples varies so much that it is virtually impossible to estimate the street dose required for detection by urinalysis. However, as discussed above that 10 mg of pure heroin taken intravenously usually produces a urine positive for morphine up to 40 h. Larger doses may be detected up to 72 h[15]. Gorodetzky and Kullberg[16,17] reported 89% of urines positive for total morphine up to 48 h using thin-layer chromatography (TLC) preceded by acid hydrolysis and XAD-2 resin extraction after a single i.v. dose of heroin at the concentration of 10 mg per 70 kg. In another similar study, Gorodetzky[18] reported 38% of urines positive for total morphine up to 40 h using enzyme multiplied immunoassay technique (EMIT) and 39% of urines positive for total morphine up to 72 h after a single i.v. dose of heroin at a concentration of 10 mg per 70 kg using radioimmunoassay technique (RIA–I^{125}). The above excretion data for morphine and its conjugates (the metabolites of heroin) in human urine proves the importance of the time of urine collection following heroin use. A sample of urine collected within the first 2 h after an "average" heroin dose may show positive results for heroin and morphine but, after 2–8 h it will show positive for morphine only. Sometimes a client honestly admits to his counselor about the recent use of heroin or of a particular drug. This statement can mislead the counselor because the client does not know the actual content of the sample ingested, although he may insist that he does. A recent report by "Analysis Anonymous", a public street drug analysis services provided by the Pharm. Chem. Research Foundation[19] revealed that over 50% of street drugs samples are not what they are alleged to be. It is, therefore, recommended that the counselor should rely more on the findings of the urine test report rather than on the statement of his client.

B. Codeine (3-methylmorphine)

Codeine is widely used for its antitussive action and is one of the common components of cough medicines such as Actifed-C Expectorant, Robitussin A-C, Phenergan Expectorant w/Codeine, Dimetane Expectorant-DC, Terpin Hydrate and Codeine Elixir, etc. It is also widely used as pain killer in combination with commonly prescribed preparations such as APC with codeine (Empirin compound with codeine), Tylenol with codeine, and also in preparations containing sympathomimetic amines such as pseudoephedrine, phenylpropanolamine (Actifed-C, Sinutab with codeine, Emprazil-C, etc.). There are total of thirty one pharmaceutial preparations listed in the 30th Edition of Physician's Desk Reference 1976 (PDR) which contain codeine as one of the active ingredients. The most common salts of codeine are codeine sulfate and codeine phosphate. Regarding its analgesic activity, it is one third as effective orally as it is parenterally. Orally a dose of 32 mg of codeine is approximately equianalgesic with one of 600 mg of aspirin, thus a combination of these two drugs leads to supra-additive effect[20].

Codeine when ingested internally is excreted as unchanged codeine, conjugated codeine, morphine (free and conjugated) and norcodeine. A small fraction (approximately 10%) of ingested codeine is demethylated to form morphine[21]. The following

metabolites have been identified in the urine of man after receiving 30 mg dose of codeine: morphine 5–17% of the injected dose, norcodeine (N-demethylated codeine) 10–21% of the total, conjugated codeine 32–51% of the total, and unchanged codeine 5–10% for a 24 h period[22]. The presence of another metabolite, non-morphine was also detected after acid hydrolysis of human urine collected for 10 h after ingestion of 10–20 mg of codeine phosphate[23]. Data from ^{14}C labeled codeine in man showed a rapid excretion of the drug, approximately one-half the dose was found to be excreted within 6 h.

Since codeine is one of the widely prescribed drugs, the urine testing procedures for admission of new clients to the various treatment programs, and for the clients who are already on various treatment programs must be able to differentiate codeine use from heroin use. The positive results for morphine obtained by immunoassay techniques such as EMIT and RIA must be confirmed by alternate procedures such as TLC and gas chromatography (GC). Immunoassay techniques can not differentiate morphine from codeine, in fact they are more sensitive to codeine than morphine. Using immunoassay techniques, the innocent client can be erroneously presumed to have abused heroin when in truth, he has not.

C. Quinine and procaine

Quinine is extensively used to dilute (adulterate or "cut") street heroin, especially in the East and Midwest (Chicago and New York City), whereas procaine (Novocaine) is a common adulterant in the West and Southwest[15]. Procaine is also used as a diluent in street cocaine, so a positive procaine test could also be due to the use of street cocaine, or also due to the use of procaine pencillin (a salt of pencillin with procaine used for gradual release of penicillin in the body). Procaine is hydrolyzed enzymatically at a very high rate with the formation of *p*-aminobenzoic acid (PABA) and diethylaminoethanol. Only 2% of the i.v. dose is excreted unchanged in the following 24 h; 90% is excreted as PABA (free and conjugated); of the total diethylaminoethanol formed, only 33% is excreted unchanged[24]. The presence of quinine and its metabolites or procaine and/or its metabolites in urine can be presumptive of heroin or other street drugs usage. In order that these indicators be used as effective tool for identifying the usage of heroin or street drugs, it is essential that the Program forbid the use of tonic water (quinine water) with alcoholic mixed drinks or any over-the-counter (OTC) medication containing quinine without prior permission. Another drug which can give positive quinine test in urine using TLC or fluorescence detection techniques is quinidine. The major clinical use of quinidine is the prevention of certain cardiac arrhythmias. In doubtful cases the counselor should require evidence of a prescription. Eight years experience with the State of Illinois Drug Abuse Programs has established the high desirability that quinine be tested in every client's urine because of its slow excretion rate and the highest sensitivity of the test. In certain populations of our clients, we have found that out of the total urines, a significant percentage of positive quinine results can be seen even when no morphine can be detected. This has been observed to occur as often as 20% of the tests in certain Programs.

Quinine excretion is markedly affected by the acid–base equilibrium of the body. It has been reported that a man receiving 500 mg of quinine orally, excreted

5.2–11% of it in urine having a pH of 7.2–7.7 and 13.5–22.5% when the urinary pH was 5–5.8[25]. Quinine is a derivative of quinoline and is metabolized by hydroxylation at the 2-position of the quinuclidine nucleus forming non-phenolic monohydroxyquinine and to a smaller extent at the 2'-position of the quinoline ring forming quinine carbostyril. It is also excreted to some extent in the unchanged form[26]. Quinine forms a highly fluorescent chromophore on treatment with sulfuric acid which forms the basis of its quantitative and qualitative detection. Quinine is non-fluorescent at a pH above 9.5 but it becomes weakly fluorescent (blue-violet) between pH 6.1 and 9.5 or strongly blue fluorescent at a pH range of 0–6 due to the formation of an oxonium group through the $-OCH_3$ substitution[27].

D. Cocaine

Cocaine is an alkaloid obtained from the leaves of *Erythroxylon Coca* or by synthesis from ecgonine; chemically it is methyl benzoyl ecgonine. It was considered to be an expensive drug, but, today it is cheaper and readily available to drug addicts. It is sold on the street under the names of Çorrine, Coke, Flake, Snow, Gold Dust, Star Dust, and Bernice. Procaine (Novocain) is a common adulterant used to dilute cocaine. Cocaine is rapidly biotransformed by the body into a major metabolite, benzoyl ecgonine and a minor metabolite, ecgonine. Fish and Wilson[28] published quantitative data on the excretion of unchanged cocaine and benzoyl ecgonine in the addicts' urine (1–9% unchanged cocaine and 49–54% benzoyl ecgonine). Due to highly water soluble characteristics of benzoyl ecgonine(the major cocaine metabolite), the abuse of cocaine remained undetected by commonly used TLC procedures. Only recently that the TLC procedures have been modified to detect the presence of benzoyl ecgonine[29–32]. Immunoassay techniques for the detection of benzoyl ecgonine are also available[33,34].

E. Central nervous system stimulants (amphetamine and congeners)

Amphetamine, methylamphetamine (methamphetamine), phenmetrazine (Preludin), diethylpropion (Tenuate), methylphenidate (Ritalin) and pipradrol are alleged to be widely abused because of their central nervous system (CNS) stimulant activity. Amphetamine, methamphetamine, phenmetrazine, and diethylpropion belong to a class of drugs known as sympathomimetic drugs (drugs acting on postganglionic adrenergic nerve endings and structures innervated by them). The subjective effects of amphetamine and congeners include a decreased sense of hunger and fatigue, an increased mental alertness, and an increased sense of well being[35]. Amphetamines are seldom used alone by addicts, but are frequently abused with barbiturates and alcohol. Ephedrine belonging to the same class is used as adulterant to dilute street amphetamines.

Amphetamine is *dl*-α-methylphenethylamine, its *d*-form is known as Dextroamphetamine and it is commonly known by its brand names as Benzedrine and Dexedrine. As discussed earlier, the execretion of unchanged amphetamine is influenced by urinary pH, being as much as 54% at pH 5 and as low as 2.9% at pH 8. About 30–40% of amphetamine is usually excreted unchanged in the urine within 48 h. After single large doses, amphetamine is slowly excreted over 5–7 days suggesting

the possibility of cumulative effects after repeated administration. Following oral administration of small doses in man, 66% of the dose was excreted in the first 24 h. In addition to the 30% of the dose excreted as unchanged, the urine contained 3% as conjugated *p*-hydroxyamphetamine, 3% as conjugated benzyl methyl ketone, benzoic acid and traces of conjugated 1-phenyl propan-2-ol[36,37]. Kaistha and Jaffe were able to detect unchanged amphetamine up to 29 h after a single oral dose of 5 mg[38].

Methamphetamine (methylamphetamine, desoxyephedrine) is *d*-N,α-dimethylphenethylamine and is commonly known by its brand name as Desoxyn. The excretion of methylamphetamine is significantly influenced by changes in the urinary pH. Under normal conditions it is mainly excreted as unchanged, only a small amount is demethylated to amphetamine. Subjective effects are more prolonged under alkaline urine conditions due to reabsorption and longer retention of the drug in the body. Under acidic conditions, the unchanged methamphetamine excretion is increased 40 times in the first 16 h[39]. Kaistha and Jaffe[38] were able to detect unchanged methamphetamine up to 23 h after a single oral dose of 5–6 mg. Immunoassay techniques* for the detection of amphetamines are also available[33,34].

Amphetamine and methamphetamine are sold on the street under various names such as Bennies, Dexies, Hearts, Pep Pills, Speed, Lid Proppers, Wake-Ups and Ups.

Phenmetrazine (3-methyl-2-phenylmorpholine) is sold under the name of Preludin, a widely prescribed drug for the treatment of obesity. Widespread misuse of this drug as anoretic agent (appetite depressant) has become very common. Although phenmetrazine seems to be less potent as CNS stimulant than amphetamine or methamphetamine, addicts achieve the desired effects by increasing the dose. The excretion of this drug is influenced by urinary pH and can be increased under acidic conditions. Most of this drug appears to be excreted in the unchanged form. Kaistha and Jaffe[38] were able to detect the unchanged drug up to 22 h after a single oral dose of 8 mg. The abuse of the drug can be easily detected using TLC as proposed by Kaistha and Jaffe[40], and Kaistha, Tadrus and Janda[41].

Diethylpropion (Tenuate, Tepanil), benzheptamine (Didrex), phendimetrazine (Dietrol, Plegine), phentermine resin and hydrochloride (Ionamin: Wilpo) belong to the same class of anoretic drugs. Among this group of drugs, diethylpropion is preferred by drug abusers next to phenmetrazine. According to Banci *et al.*[42], the oral administration of diethylpropion to human volunteers gave no detectable amounts of unchanged drug in the urine. The following metabolites were present: 1-phenyl-2-diethylamino-1-propanol in *threo* form is one of the main metabolites; 2-ethylaminopropiophenone(1-phenyl-2-ethylamino-1-propanone) is also excreted in equal amounts; *threo*-amino alcohol (1-phenyl-2-ethylamino-1-propanol) is excreted in small amounts; and 1-phenyl-2-amino-1-propanol (both in *threo* and *erythro* forms) is excreted in small amounts. In man, the metabolic pathway is N-de-ethylation and reduction while in rabbit, N-de-ethylation is the only metabolic pathway. In rabbit urines no amino alcohols were found while 2-ethylaminopropiophenone was always present. Schreiber *et al.*[43] studied the metabolism of [1-^{14}C]diethylpropion in humans and reported that they were able to identify twenty-one metabolic products including hippuric acid (27% of the radioactivity excreted in the urine), mandelic acid (only

* EMIT can detect amphetamine and/or methamphetamine as a class without any differentiation; RIA amphetamine antibody and radiolabeled antigen as currently available are specific for the detection of amphetamine only and can not detect methamphetamine.

0.3%) and benzoic acid (3.4%). According to these authors, hippuric acid and mandelic acid are the products of oxidative cleavage.

Methylphenidate (Ritalin) is α-phenyl-2-piperidine acetic acid methyl ester. It is a mild CNS stimulant and is used as antidepressant. As a cortical stimulant, it is more potent than caffeine but less potent than amphetamine. Since the toxic effects are less pronounced and occur less frequently, it has more abuse potential. The metabolism and disposition of methylphenidate using ^{14}C-labeled drug in human subjects were studied by Farraj *et al.*[44]. After oral administration, 50 and 90% of the ^{14}C was excreted in urine in 8 and 48 h, respectively. The main urinary metabolite was the de-esterified product, ritalinic acid (RA), which accounted for 80% of the dose. In addition, oxo-ritalinic acid (oxo-RA, the lactam of ritalinic acid) and *p*-hydroxy ritalinic acid (*p*-OH RA) accounted for 2.5 and 1.5% of the radioactivity. No appreciable amounts of unchanged drug were detected at low doses (20 mg), however, at a higher dose (100 mg), unchanged drug was detected (2.2–2.4% of the dose). Schubert[45] observed high concentrations of unchanged methylphenidate (up to 40 μg/ml) in urine of subjects who had ingested large doses. Also, the State of Illinois laboratory has been able to detect unchanged methylphenidate by a TLC detection procedure as previously proposed[40,41] in the urines of drug abusers alleged to have ingested this drug.

Pipradrol (Meratran, Alertol) is α,α-diphenyl-α-piperid-2-yl methanol. This is a mild CNS stimulant and is abused if available in illicit market. Pipradrol is stated to be rapidly absorbed after oral administration and is excreted in the urine as metabolites which have not been identified. Due to its simple chemical nature, the drug is likely to be excreted unchanged in significant amounts.

F. Sedative hypnotics

Most of the modern sedative hypnotics are general depressants and can be classified according to their chemical structure and therapeutic uses. The most widely used class of drugs as sedative hypnotics are diureides (barbiturates); piperidiendione derivatives (glutethimide and methyprylon); 2,3-disubstituted quinazalones (methaqualone); carbamic acid esters of glycols (meprobamate); tertiary acetylenic alcohols (ethychlorvynol) and benzodiazepine compounds (chlordiazepoxide, diazepam, and flurazepam).

a. Barbiturates (diureides)

The barbiturates are ureides formed from the combination of urea and various organic acids. Acids with two carboxyl groups may react with urea to form cyclic diureides. The most commonly abused barbiturates are phenobarbital, amobarbital, pentobarbital, and secobarbital. Phenobarbital is long acting; amobarbital and pentobarbital are intermediate acting: secobarbital is short acting. Absorption of barbiturates occurs from the stomach and small intestine after their oral ingestion. The short-acting barbiturates are absorbed rapidly; the long-acting barbiturates are absorbed slowly, thus the time period during which barbiturates are detectable in urine is variable. As a class, a single therapeutic dose (30–60 mg) is usually detectable for 24–40 h. Jaffe and Kaistha[38] were able to detect unchanged phenobarbital and secobarbital using TLC technique for 24–40 h after single therapeutic doses of 30 and 60 mg, respectively. When larger doses are abused, these drugs may be detected for 96 h or longer[15]. Since many prescription items contain phenobarbital as one of the several ingredients, the client showing positive barbiturates test must be examined for

the possibility of interference from the ingredients of such prescribed medications. In addition, the client must be interrogated for the prescribed use of anticonvulsant drugs such as diphenylhydantoin (Phenytoin, Dilantin) and primidone (Mysoline). If the client is using diphenylhydantoin as anticonvulsant drug and the testing laboratory has reported a positive barbiturate test, the laboratory should be asked for the re-check to differentiate between phenobarbital and diphenylhydantoin. However, it may be pointed out that the concurrent use of phenobarbital and diphenylhydantoin to epileptic patients is quite common for the effective control of the epileptic seizures. Immunoassay techniques for the detection of barbiturates are also available[33,34].

The urines of drug addicts quite often are positive both for barbiturate and morphine because pushers sometimes sell street heroin adulterated with phenobarbital. Mysoline (primidone), an anticonvulsant for epileptics, is oxidized to phenobarbital in the human body[46]. Primidone is an analogue of phenobarbital in which oxygen at position 2 of the barbiturate ring is replaced by hydrogen. It appears that the action of primidone is due to its metabolic conversion to phenobarbital. Therefore, the urine specimen of the client who has ingested this drug will be positive for phenobarbital although none was taken. Phenobarbital in the human body is metabolized to its inactive form, hydroxyphenobarbital (5-ethyl-5-hydroxyphenylbarbituric acid) by *p*-hydroxylation of the aromatic ring. This inactive form is excreted as ethereal sulfate (40%) and as the glucuronide (less than 10%). After the administration of a single dose of 300 mg in man, the unchanged drug was detected in the urine for 17–28 days and *p*-hydroxyphenobarbital for 12–24 days using paper chromatography[47]. Methylphenobarbital (Mephobarbital, Mebaral) is 5-ethyl-1-methyl-5-phenylbarbituric acid and is a milder hypnotic than phenobarbital. The ingestion of this barbiturate will also result in the excretion of phenobarbital in the urine due to N-dealkylation of the methyl group[48].

Amylobarbitone (Amytal) is metabolized, like phenobarbital, to its inactive form, hydroxyamylobarbital.

Pentobarbital is metabolized in the liver by hydroxylation to 5-ethyl-5-(3-hydroxy-1-methylbutyl)barbituric acid (hydroxypentobarbital) and by oxidation to pentobarbital carboxylic acid[49]. Both of these metabolites and unchanged pentobarbital have been found in the urine.

Thiopentone (Thiopental) which is sometimes i.v. administered for the production of complete anesthesia of short duration could also result in the formation of pentobarbital due to desulphurisation of the sulfur group[50]. Secobarbital (Quinalbarbitone) is excreted as hydroxyquinalbarbitone and quinalbarbitone carboxylic acid. Only a small amount of ingested quinalbarbitone is excreted unchanged in the urine[51]. The only barbiturate which is resistant to the metabolic processes of human and animal bodies is barbital; it is excreted practically unchanged in the urines[52].

Barbiturates are known in street language as Barbs, Red Devils, Yellow Jackets, Phennies, Peanuts, Blue Heavens, Candy, Downs and Goof Balls.

Diphenylhydantoin is metabolized by two main routes. It undergoes automatic hydroxylation to 5-(*p*-hydroxyphenyl)-5-phenyl hydantoin and about 44–62% of the dose appears in the urine as the conjugated hydroxy metabolite. It also undergoes hydrolysis of the hydantoin ring to α-aminodiphenyl-acetic acid. Ring fission to hydantoic acid may also occur. In man and dog approximately 1–4% of the administered dose is excreted unchanged in the urine over a 3-day period, and about 10–27% appears as α-aminodiphenylacetic acid[53,54].

b. Glutethimide and methyprylon

Glutethimide (Doriden) and methyprylon (Noludar) are piperidinedione derivatives with actions closely resembling those of barbiturates. The hypnotic effects of glutethimide and methyprylon are similar to secobarbital. Glutethimide is metabolized by hydroxylation in the glutarimide ring and in the ethyl side-chain and the metabolites formed are excreted as conjugates. The hydroxylated ethyl group in the side chain can be lost to form α-phenylglutarimide[55]. Methyprylon is excreted in the urine as glucuronides and unconjugated metabolites, only 3% of the orally administered dose appears in the urine unchanged. About 3% appears in the urine as the dehydrogenated product (3,3-diethyl-2,4-dioxo-5-methyl-tetrahydropyridine[56].

c. Methaqualone

Methaqualone (Quaalude), 2-methyl-3-*o*-tolyl-4-(3H)-quinazolinone, is similar to barbiturates in its hypnotic effects and appears to have no advantage over the barbiturates. It does not possess significant analgesic activity although it enhances the analgesic action of codeine. Potentiation of the hypnotic effects occurs when this drug is used concomitantly with barbiturates, ethanol, chlorpromazine, reserpine, meperidine and other opiates[57]. Since the implementation of federal restrictions on the prescription and distribution of methaqualone, its abuse has decreased substantially within the past 2 years. The drug has been popular among its users as "Love Pill". It is alleged to be used during daytime when the addicts can not obtain other drugs as it gives them a buzz or thrill not obtained from other hypnotics[58]. Preuss *et al.*[59] isolated some thirteen metabolites from urine of human subjects who had ingested methaqualone. These studies showed that biotransformation of methaqualone is largely oxidative in nature, not affecting the basic structure. The benzene ring and the methyl groups in position 2 and 2′ are hydroxylated. These findings have been corroborated by Bonnichsen *et al.*[60,61] by the combined use of gas chromatography–mass spectrometry (GC–MS). Recently Permisohn *et al.*[62] reported a GC determination of five principal monohydroxylated metabolites of methaqualone in human urine. The metabolites reported were 2-hydroxymethyl methaqualone, 2′-hydroxymethyl methaqualone, 3′-hydroxymethaqualone, 4′-hydroxymethaqualone and 6-hydroxy-methaqualone (4′-hydroxymethaqualone was the major component, 3′-hydroxymethaqualone and 2′-hydroxymethaqualone were next in order). Controlled time study from a volunteer who had ingested a 300-mg dose of methaqualone showed unchanged methaqualone at the level of 0.25 μg/ml of urine from a specimen collected after 24 h. In another time study, a total of 350 μg or 0.1% of the dose was excreted as the unchanged drug in the urine during the first 24 h after the ingestion of a therapeutic dose. In one urine specimen from the U.S. Air Force Europe (USAFE) Drug Abuse Detection Laboratory, the authors[62] found unchanged methaqualone at a concentration of 1.24 μg/ml of urine. Since it is common practice for the drug abusers to consume multiple doses of methaqualone, it is possible to find the presence of unchanged methaqualone at a concentration of 1 μg/ml of urine. Burnett *et al.*[58], and Sleeman *et al.*[63] have reported TLC identification of methaqualone and/or its metabolites in urine. A RIA technique has been reported by Berman *et al.*[64]. Bost *et al.*[65] recently suggested that RIA may be used for the presence of methaqualone or one of its metabolites while GC may be used when the concentration of the parent drug is required.

d. Meprobamate

Meprobamate is a carbamic acid ester of glycol sold under the brand names of Miltown and Equanil. It has been a quite popular drug for the treatment of anxiety. The drug has been used as a day-time sedative and as a hypnotic in the treatment of insomnia. Meprobamate is readily absorbed from the gastrointestinal tract. About 10% of the unchanged drug can be detected in the urine within 24 h after ingestion. Most of the remaining 90% is excreted in the urine as an oxidized derivative, hydroxymeprobamate, and a glucuronide[66]. A single dose of 400 mg is detectable in the urine for 48 h and maximum serum levels occur 2 h after ingestion[67]. Carisoprodol (Rela, Soma) used as a muscle relaxant drug is excreted in the urine of the dog in small quantities as hydroxymeprobamate and meprobamate in addition to hydroxycarisoprodol[68]. Tybamate (Benvil, Solacen) used as a minor tranquillizer and chemicaliy related to meprobamate is excreted in smaller amounts as hydroxymeprobamate in addition to hydroxytybamate, a major metabolite of tybamate[69]. The presence of meprobamate in urine can be detected using TLC techniques. Detection reagents employed to form chromogenic reactions with meprobamate and other carbamates (methocarbamol, ethinamate, carisoprodol and tybamate) are furfural 10% in alcohol, and vanillin in sulfuric acid[70].

e. Ethylchlorvynol

Ethylchlorvynol (Placidyl) is a tertiary acetylenic alcohol having a β-chlorovinyl group. The drug is an effective hypnotic with a short duration of action. It has anticonvulsant and muscle-relaxing properties. Approximately 10% of the ingested drug is excreted unchanged in the urine within the first 24 h, thereafter the amount of unaltered drug diminishes rapidly[71].

Chlordiazepoxide, diazepam and flurazepam are discussed under benzodiazepines.

G. Drugs used in the treatment of psychoses, anxiety and depression

Until the late 1950s there was no widely accepted pharmacological treatment for depression except psychotherapy for mild depression and electro-shock therapy for severe depression. The big break-through came in the early 1950's when chlorpromazine (Thorazine) was synthesized. The phenothiazines as a class and especially chlorpromazine are among the most widely prescribed drugs for treatment of psychiatric patients and treatment of nausea and vomiting.

Drugs used in the treatment of anxiety and neurotic conditions are benzodiazepine compounds, meprobamate, and some barbiturates and non-barbiturate sedatives which have already been discussed. Drugs employed in the treatment of depression are monoamine oxidase inhibitors (MAO) such as iproniazid, isocarboxazid (Marplan), nialamide (Niamid), phenelzine (Nardil) and tranylcypromine (Parnate); and dibenzazepine tricyclic antidepressants such as imipramine, amitriptyline, desipramine, nortriptyline, and doxepin.

a. Phenothiazine derivatives

Phenothiazine derivatives used in psychiatry have a 3-carbon bridge between

the ring and side-chain nitrogen atoms *e.g.*, chlorpromazine (Thorazine, Largactil), triflupromazine (Vesprin), fluphenazine (Permitil, Prolixin), prochlorperazine (Compazine) and trifluoperazine (Stelazine). Methdilazine, although it has three carbons between the nitrogens but lacks significant antipsychotic action. Thioridazine (Mellaril) having a piperidine moiety in the side chain is also a widely prescribed tranquilizer. The phenothiazine drugs are well absorbed from the gastrointestinal tract and from parenteral sites. After absorption, the phenothiazines are rapidly distributed in all body tissues. Approximately half of the metabolites of commonly used phenothiazines are found in the urine and the rest in the faeces. The known metabolic pathways of phenothiazines are (i) sulfoxidation to a sulfoxide which then may undergo additional metabolism to the sulfone; (ii) demethylation of the dialkylamino group; (iii) hydroxylation and subsequent conjugation with glucuronic acid; (iv) oxidation of the dialkylamino group to an N-oxide. Combinations of these reactions give rise to numerous metabolites, *e.g.*, chlorpromazine gives rise to more than twenty metabolites in urine.

In man urinary excretion of chlorpromazine varies from 1–20% of the daily dose administered; 20% of the metabolites are non-conjugated sulfoxides and less than 1% is free chlorpromazine; remaining are hydroxylated products and their conjugates, N-oxides, demethylated derivatives and unknown products[72]. In case of thioridazine, an average of 10.4% of the administered dose may be recovered unchanged within 24 h in the urine[73]. Triflupromazine is converted into demethyltriflupromazine, a demethylated derivative having antidepressant properties resembling imipramine[74].

b. Benzodiazepine compounds

Benzodiazepine derivatives such as chlordiazepoxide (Librium), diazepam (Valium), and oxazepam (Serax) are widely prescribed drugs not only for the treatment of anxiety but also for skeletal muscle relaxation and combating alcoholism. These drugs are not excreted in the urine in the free unchanged form to any appreciable extent. The methods for their detection have to be based on the identification of their metabolites. Since chlordiazepoxide (Librium) and particularly diazepam (Valium) are alleged to be widely abused drugs, the information on their biotransformed products will be highly desirable.

(i) Chlordiazepoxide (Librium). Chlordiazepoxide is well absorbed from the gastrointestinal tract. Plasma levels of 0.48 μg to 1.27 μg of drug per ml have been observed after oral ingestion of 10–30 mg of chlordiazepoxide. The drug has a half-life of 16–27 h in man after a single 30-mg i.v. dose; this half-life could be longer in individuals having a slower rate of metabolism. The sedative effects appear within 3–30 min following i.v. administration[75]. The metabolic fate of chlordiazepoxide in man has been studied by Koechlin *et al.*[76] and Schwartz *et al.*[77,78]. The drug is first demethylated to N-demethylchlordiazepoxide (7-chloro-2-amino-5-phenyl-3H-1,4-benzodiazepin-4-oxide) which is further metabolized to lactam, the demoxepam (7-chloro-1,3-dihydro-5-phenyl-2H-1,4-benzodiazepin-2-one-4-oxide) and desoxydemoxepam by liver. Only small amounts of unchanged drug appear in the urine[79]. Excretion continues for several days at a low level after the administration is discontinued[76].

The major urinary metabolite is demoxepam with smaller amounts of the N-demethylchlordiazepoxide. Koechlin *et al.*[76] presented evidence that the urinary

metabolites of [^{14}C]-chlordiazepoxide in man also included N-(2-amino-5-chloro-α-phenylbenzylidene)glycine N-oxide (opened lactam). Kimmel and Walkenstein[80] and Schwartz *et al.*[81] reported three additional metabolites of demoxepam in dog's and rat's urines. One was oxazepam (1% of the dose) and other two were phenols [7-chloro-1,3-dihydro-5-(4-hydroxyphenyl)-2H-1,4-benzodiazepin-2-one-4-oxide and its deoxy form in which 4-oxide (N-oxide) is absent]. These findings were further confirmed on dog by Schwartz *et al.*[82] by using labeled demoxapam. Chlordiazepoxide, N-demethylchlordiazepoxide and demoxepam may be measured in blood and plasma spectrophotofluorometrically by the methods described by Schwartz and Postma[77] and Koechlin and D'Arconte[83]. A quantitative colorimetric procedure for the total assay of urinary metabolites is available if needed[75,79]. The method is based on the acid hydrolysis of these metabolites into corresponding aminobenzophenones which are then coupled with the Bratton-Marshall reagent[84] for primary aromatic amines. Kokoski, Hamner and Shiplet[85] have reported a TLC procedure for the detection of metabolites of chlordiazepoxide, diazepam and oxazepam based on the above principle. Recently, Vandemark and Adams[86] proposed a GC procedure using nitrogen detector for the determination of unchanged benzodiazepines in serum. An immunoassay procedure for the detection of unchanged chlordiazepoxide in urine has been reported by Haden *et al.*[87] using a mixture of antibenzodiazepin γ-globulin and enzyme substrate. Syva has propheted EMIT reagents for the detection of benzodiazepines as a class for the drugs excreted in urine as oxazepam.

(ii) Diazepam (Valium). Diazepam is one of the most commonly abused drugs at the present time. The drug is absorbed from the gastrointestinal tract when ingested orally; when injected it is absorbed directly into blood stream. Blood levels of diazepam ranged from 0.137–0.189 μg/ml at 1–1.5 h after oral ingestion and from 0.294–0.574 μg/ml at 1–5 min after i.v. administration of a single 10-mg dose. The demethyldiazepam maximum blood levels of 0.026–0.037 μg/ml were reached at 30–48 h[75]. Metabolism of diazepam has been discussed in depth by Kaplan *et al.*[88], Greenblatt and Shader[89], de Silva and Puglisi[90], Schallek *et al.*[91], Blacow[92], and Schwartz *et al.*[93]. The drug is completely biotransformed in the body through demethylation, hydroxylation and conjugation processes. No measurable amounts of unchanged diazepam are excreted in the urine. The major metabolites identified are (i) N-demethyldiazepam or nordiazepam (7-chloro-1,3-dihydro-5-phenyl-2H-1,4-benzodiazepin-2-one), (ii) 3-hydroxy-diazepam (7-chloro-1,3-dihydro-3-hydroxy-1-methyl-5-phenyl-2H-1,4-benzodiazepin-2-one), (iii) oxazepam (7-chloro-1,3-dihydro-3-hydroxy-5-phenyl-2H-1,4-benzodiazepin-2-one), (iv) oxazepam glucuronide measured as yellow colored 2-amino-5-chlorobenzophenone (ACB) derivative after acid hydrolysis and (v) 3-hydroxydiazepam glucuronide measured as 2-methylamino-5-chloro-benzophenone (MACB) derivative after acid hydrolysis. The conjugated demethyldiazepam accounted for approximately 2.5 to 9% of the administered dose. The major urinary metabolite is oxazepam glucuronide with small amounts of 3-hydroxydiazepam glucuronide[94]. Both of these can be seen as ACB and MACB after acid hydrolysis. The acid hydrolysis reaction was used by de Silva *et al.*[95,96] in their studies on blood levels of diazepam and metabolites. Recently Meola and Brown[97] reported a TLC procedure involving enzymatic hydrolysis of oxazepam glucuronide. An immunoassay procedure has also been reported by Haden *et al.*[87] for the detection of diazepam, oxazepam and N-demethyldiazepam in urine but it is not specific for diazepam and its metabolites.

It is interesting to know that three major metabolites of diazepam, *i.e.*, 3-hydroxydiazepam, desmethyldiazepam and oxazepam exhibit significant pharmacological activity. The clients who are prescribed diazepam (Valium) should be advised against the simultaneous ingestion of alcohol and other CNS depressants. They should also be cautioned against driving automobiles or operating dangerous machinery until it is known that they do not become drowsy or dizzy on diazepam or oxazepam therapy.

(iii) Oxazepam (Serax). Oxazepam, the major metabolite of diazepam is on the market under the trade name of Serax. Its clinical use is indicated for the management and control of anxiety, tension, agitation, irritability and related symptoms. The drug is likely to be excreted as oxazepam glucuronide, a single major metabolite.

(iv) Flurazepam (Dalmane). Flurazepam is a non-barbiturate hypnotic agent gaining a widespread usage as a "sleeping pill". It is structurally related to the benzodiazepine tranquilizers discussed above. Flurazepam hydrochloride is chemically 7-chloro-1-(2-diethylaminoethyl)-5-(2-fluorophenyl)-1,3-dihydro-2H-1,4-benzodiazepin-2-one dihydrochloride. It is used for all types of insomnia characterized by difficulty in falling asleep, frequent noctural awakenings, or for patients with poor sleeping habits. In hospitals, this drug appears to be widely prescribed in acute or chronic medical situations requiring restful sleep. Flurazepam hydrochloride is rapidly absorbed from the gastrointestinal tract and undergoes extensive biotransformation to a number of metabolites which are measurable in blood and urine[98–100]. Two metabolites reported in blood and urine are N-1-hydroxyethylflurazepam [7-chloro-1-(2-hydroxyethyl)-5-(2′-fluorophenyl)-1,3-dihydro-2H-1,4-benzodiazepin-2-one] and N-1-dealkylflurazapam [7-chloro-5-(2′-fluorophenyl)-1,3-dihydro-2H-1,4-benzodiazepin-2-one]. The major urinary metabolite is N-1-hydroxylated flurazepam and the primary metabolite found in the blood is N-1-dealkyl flurazepam. Detection procedures for flurazepam and its metabolites in blood and/or urine using spectrofluorometry, fluorodensitometry and electron capture gas-liquid chromatography (GLC) have been reported by de Silva *et al.*[101–103]. A TLC identification procedure using ion-exchange paper extraction[104] and XAD-2 resin column extraction for the detection of flurazepam and its primary urinary metabolite, N-1-hydroxylated flurazepam has been proposed by Manno *et al.*[105]. Clients ingesting flurazepam should be cautioned against the simultaneous use of alcohol or other CNS depressants. Drugs having hypnotic properties should not be administered simultaneously to avoid additive or synergistic effects.

(v) Clonazepam (Clonopin). Clonazepam is an oral antiepileptic agent structurally resembling other benzodiazepine compounds. Chemically it is 5-(2-chlorophenyl)-1,3-dihydro-7-nitro-2H-1,4-benzodiazepine-2-one. The drug apparently undergoes extensive biotransformation in the body since less than 0.5% of a single 2-mg oral dose was recovered in the urine as intact drug in 24 h[75,106]. The major metabolic pathway is by the reduction of the nitro group to the amine which is then acetylated to the acetamide[107,108]. Hydroxylation at the C-3 position also occurs, resulting in the elimination of these metabolites as their glucuronide and/or sulfate conjugates. A GLC procedure using an electron capture detector has been reported for the detection of intact clonazepam and flunitrazepam in blood and urine[109,110]. The procedure involves the extraction of these compounds into an organic solvent at

a pH of 9.0 and then their conversion to respective aminobenzophenones by acid hydrolysis. A differential pulse polarographic assay for the determination of the major urinary metabolites of clonazepam was also presented[109].

(vi) Monoamine oxidase inhibitors (MAO). The drugs classified as monoamine oxidase inhibitors have in common the ability to block oxidative deamination of naturally occurring amines. Because of toxicity, drugs like iproniazid, pheniprazine and etryptamine are no longer available. At present, the drugs marketed for use in psychiatric depression are isocarboxazid (Marplan), nialamide (Niamid), phenelyzine (Nardil) and tranylcypromine (Parnate). The first three drugs are derivatives of hydrazine; tranylcypromine is a non-hydrazine MAO inhibitor resulted from the cyclization of amphetamine. All the currently available MAO inhibitors are readily absorbed when given by mouth. They have a major effect on the enzymes in the liver. They interfere with the metabolic degradation of barbiturates, aminopyrine, acetanilide, cocaine and meperidine.

c. Dibenzazepine and tricyclic antidepressants

The dibenzazepine derivatives most widely prescribed are impramine, amitriptyline and doxepin.

(i) Imipramine. Imipramine is 5-(3-dimethylaminopropyl)-10,11-dihydro-5H-dibenz[*b,f*]azepine and is sold under various brand names such as Tofranil, Imavate, Presamine and Pramine. It is rapidly absorbed from the gastrointestinal tract and is largely bounded to plasma proteins. Imipramine is rapidly metabolized in the body by N-demethylation to yield desipramine and by hydroxylation followed by glucuronide conjugation. After administration of large doses, only small amounts of unchanged drug are excreted[111,289]. It has been proposed that the antidepressant action of imipramine is due to desipramine. Imipramine and desipramine can be quantitatively estimated in biological fluids and tissues after extraction into an organic solvent followed by direct spectrophotometry[112] or by spectrophotometric modification[113] of the urinary screening test involving the use of Forrest reagent[114] (potassium dichromate–perchloric acid reagent). Wallace and Biggs[115] proposed a similar quantitative method using cerium sulfate in sulfuric acid.

Imipramine (unchanged if any) and its metabolite desipramine in urine can also be detected using TLC by the spraying technique proposed by Kaistha *et al.*[41].

The concomitant use of monoamine oxidase inhibiting drugs is contraindicated. The use of preparations such as decongestants and local anesthetics, which contain any sympathomimetic amine (*e.g.*, adrenalin and noradrenalin) should be avoided since these can potentiate the effects of catecholamines.

(ii) Desipramine. Desipramine is a metabolite of imipramine resulting from N-demethylation. It is marketed under the brand names of Norpramin and Pertofrane. Chemically it is 10,11-dihydro-5-[3-(methylamino)propyl]-5H-dibenz-[*b,f*]azepine, it differs from the parent substance by having only one methyl group on the side chain nitrogen. It is not a monoamine oxidase inhibitor and does not act primarily as a CNS stimulant. The drug is metabolized in the liver and approximately 70% is excreted in the urine[116].

(iii) Amitriptyline. Amitriptyline is a dibenzocycloheptadiene derivative, chemically it is 10,11-dihydro-5-(3-dimethylaminopropylidene)-5H-dibenzo[*a,d*]cycloheptene. It is sold under the brand names of Elavil, Etrafon and Triavil (the last two

preparations are mixture of amitriptyline and perphenazine). Amitriptyline is rapidly metabolized in the body by demethylation, hydroxylation and conjugation with glucuronic acid. Most of these metabolic reactions are thought to occur in the liver. Nortriptyline and 10-hydroxyamitriptyline are the demethylated and hydroxylated metabolites of amitriptyline. According to Forbes *et al.*[117], analysis of the urine collected for 4 days after a volunteer had ingested 100 mg of the drug indicated an excretion of 1% of unchanged drug.

Forbes *et al.*[117], Wallace and Dahl[118] and Sunshine and Baumler[119] have described spectrophotometric methods for the quantitative determination of amitriptyline and/or its principal metabolites in biological materials including urine. A GC procedure for the determination of total metabolites and unchanged drug has been described by Gard *et al.*[120]. A TLC procedure applicable to biological materials has also been reported[121].

(iv) Nortriptyline. Nortriptyline is a demethylated metabolite of amitriptyline. It is marketed under the brand name of Aventyl HCl for the relief of symptoms of depression especially of endogenous origin. The drug is metabolized to 10-hydroxynortriptyline[122] which could be excreted free as well as in conjugated form. Quantitative spectrophotometric techniques have been reported by Amundson and Manthey[122] and by Wallace and Dahl[118] for the determination of nortriptyline and 10-hydroxynortriptyline in biological materials.

(v) Doxepin. Doxepin is one of the dibenzoxepin tricyclic compounds, chemically it is an isomeric mixture of 11-(3-dimethylaminopropylidene)-6,11-dihydrodibenz[*b*,*e*]-oxepin hydrochloride. It is sold under the brand names of Sinequan and Adapin. It provides antidepressant as well as antianxiety effects. Distribution and metabolism of doxepin has been studied in detail in rats and dogs by Hobbs[123] and in rats, dogs and humans by Kimura *et al.*[124]. TLC of the urine extracts, brain and liver indicated a multiplicity of components. Demethyl doxepin, doxepin-N-oxide, hydroxy doxepin and its glucuronide were identified as predominant metabolites of doxepin besides unchanged drug. In addition, demethyl hydroxy doxepin and didemethyl doxepin were also identified by Hobbs[123]. Devriendt *et al.*[125] described colorimetric, spectrophotometric and spectrophotofluorometric methods for the determination of doxepin and some of its metabolites in serum and in urine. Randolph *et al.*[126] reported a spectrophotometric method for measuring doxepin and some of its metabolites in human urine. The method involved oxidation of these compounds to a neutral ketone by treatment with potassium permanganate. However, according to Curry[127] amitriptyline gives an identical UV spectrum; diazepam and chlordiazepoxide also interfere. A GC procedure for the determination of doxepin in human urine was reported by Dusci and Hackett[128]. Doxepin can also be detected using TLC by the spraying technique described by Kaistha *et al.*[41].

d. Psychotogenic and psychotomimetic drugs

Drugs belonging to this group have the capacity to induce disturbances of mood, thinking, abnormalities of perception including hallucinations. There is no sharp line dividing them from other classes of drugs. This group of drugs with diverse chemical structures have the ability to produce psychotic states closely resembling the disturbances seen in the naturally occurring psychoses. Psychotomimetic drugs derived from various plants have been used from time immemorial for religious purposes.

(i) Mescaline. Mescaline, an active principle of peyote, was isolated in 1896 from the peyote cactus *Lophophora williamsii.* The drug was named after the Mescalero Apaches of the Great Plains who had developed a religious peyote rite. Chemically it is 3,4,5-trimethoxyphenethylamine. It is known to cause unusual psychic effects and visual hallucinations. The effects of a single full dose may persist for about 12 h. Mescaline is readily absorbed from the gastrointestinal tract. It is metabolized partly by oxidative deamination to 3,4,5-trimethoxyphenylacetic acid; 3,4-dihydroxy-5-methoxyphenylacetic acid is found to be excreted as a glutamine conjugate[129]. Charalampous *et al.*[130] found that human subjects given mescaline orally excreted an average of 26.2% of the dose as free 3,4,5-trimethoxyphenylacetic acid. This metabolic product is pharmacologically inactive. Mescaline in street samples and in urine (unchanged) can be detected using the TLC technique of Kaistha *et al.*[41].

(ii) Lysergic acid diethylamide (LSD). The unusual psychological effects of this compound were discovered by Hofmann in 1943. Chemically it is (+)-N,N-diethyl-lysergamide (brand name is Delysid). LSD is sold on the street under the slang names of acid, sugar, trips, cubes and Big D. It is one of the most potent known drugs and doses as low as 20 to 25 μg can produce effects in susceptible individuals. The significant effects are almost entirely upon the CNS. Individuals ingesting this drug exhibit marked changes in mood, become quite emotional, can laugh or cry with only slight provocation. Perceptual changes such as distortions, hallucinations, color changes, time perception, movement of objects, etc., constitute the most dramatic effects produced by the drug. Readers interested in knowing the detailed pharmacological effects of this drug are advised to refer to the *Pharmacological Basis of Therapeutics* edited by Goodman and Gilman[131]. The drug is rapidly absorbed after oral administration and is distributed widely throughout the body. Axelrod *et al.*[132] found that monkeys excreted less than 1% as unchanged drug in urine in 24 h after a single i.v. dose of lysergide. Seventy percent of the labelled lysergide was present in the bile and intestine at the end of 12 h[133]. Three metabolites of lysergide have been found in the bile, one of which is 2-oxy-LSD[134]. Hydroxylation at the 12-position and conjugation with glucuronic acid appear to be one of the possible routes. The rat bile was found to contain hydroxylysergide and a hydroxy-iso-lysergide as conjugates of glucuronic acid[135]. Axelrod *et al.*[132], and Upshall and Wailling[136] described a fluorometric quantitative procedure for the determination of lysergide in biological specimens. Faed and McLeod[137] reported a quantitative fluorometric procedure for the determination of LSD in human urine. The procedure involved enzymatic hydrolysis, extraction of the drug into an organic solvent followed by paper chromatography. The final step involved inactivation of the fluorescence of lysergide or its derivative in the chromatogram eluates by UV irradiation. The method can be specific only if the solvent used for paper chromatography could separate ergot alkaloids and methysergide metabolites of lysergide.

RIA techniques for the determination of lysergide in biological fluids have been reported by Taunton-Righby *et al.*[138], and Loeffler and Pierce[139]. Quantities of lysergide as low as 1 ng/ml can be determined in plasma, serum and urine. The method is not specific for lysergide as structurally similar ergot alkaloids such as ergonovine, methylergonovine and ergotamine cross-react with antibody.

(iii) STP. STP, also known as DOM, is α-methyl-2,5-dimethoxy-4-methyl-phenethylamine (2,5-dimethoxy-4-methylamphetamine). The compound is chemically

related to amphetamine and to mescaline. In the drug experimenting culture, the letters "STP" refer to Serenity, tranquillity and peace. The effects of STP have been recorded as dry mouth, dilatation of the pupil of the eye, blurred vision, multiple images, increased pulse rate and blood pressure, hallucinations, nightmares, mental disorder and loss of consciousness[139a]. According to Snyder and Faillace[139b], oral doses greater than 3 mg caused pronounced hallucination effects lasting about 8 h similar to those produced by hallucinogenic doses of LSD, mescaline, and psilocybin. STP is about one-thirtieth as potent as LSD, but 100 times more potent than mescaline. Biotransformation of STP has been studied in detail in rats[139c]. The major pathway is the hydroxylation of the 4-methyl group to 1-(2,5-dimethoxy-4-hydroxyphenyl) 2-aminopropane which in turn is oxidized to 1-(2,5-dimethoxy-4-carboxyphenyl) 2-aminopropane. A trace amount of 1-(2,5-dimethoxy-4-methylphenyl) 2-propanone is also excreted in addition to the unchanged STP (about 8% as unchanged). Excretion of STP and its metabolites is nearly complete in 24 h after administration of the drug[139d,e]. About 20% of the ingested dose in humans is excreted in the urine within 24 h[139c]. STP in street samples can be detected using the TLC spraying technique of Kaistha *et al.*[41].

(iv) MDA. MDA is methylenedioxyamphetamine. It is one of the illicit preparations frequently adulterated with amphetamine-type drugs sold on the street. In a number of cases where the cause of death was officially attributed, directly or indirectly, to MDA poisoning, the drug was detected in the urine[139c].

(v) PMA. PMA is α-methyl-*p*-methoxyphenethylamine (also known as *p*-methoxyamphetamine). It is one of the most potent hallucinogens tested with the exception of LSD. An average of 6.7% of the administered dose was excreted in the urine when given to human subjects[139c].

(vi) TMA, TMA-2 and TMA-3. Trimethoxyamphetamine (TMA) is α-methyl-3,4,5-trimethoxyphenethylamine and is a α-methyl homologue of mescaline. Doses of 0.8–2 mg/kg in human volunteers produced fairly vivid hallucinations similar to those produced by mescaline[139f]. TMA-2 is 2,4,5-trimethoxyamphetamine. In man it is a psychotomimetic agent and is about 17 times more potent than mescaline[139c]. In *in vitro* studies with rat and rabbit liver homogenates, the compound was metabolized primarily by O-demethylation[139g]. TMA-3 is 2,3,4-trimethoxyamphetamine. This is an isomer of 2,4,5-trimethoxyamphetamine and it does not possess psychotropic effects[139c]. In *in vitro* studies with rat and liver homogenates, the compound was metabolized by O-demethylation[139g].

(vii) DET. DET is 3-(2-diethylaminoethyl) indole, also called diethyltryptamine. Its pharmacological effects closely resemble to those of LSD. It is metabolized to 6-hydroxydiethyltryptamine (6-HDET) in the liver and this metabolite is excreted in the urine partly free and partly conjugated with glucuronic acid[139h]. There is some evidence that this metabolite is 5–6 times more psychologically active than DET.

(viii) DMT. DMT is 3-(2-dimethylaminoethyl) indole, also called dimethyltryptamine. It is an active principle obtained from the seeds and leaves of *Piptadenia peregrina*. Its effects on the mental state are similar in some respects to those produced by LSD. However, DMT psychosis appears somewhat more rapidly than LSD and is of shorter duration. It is not effective when taken orally[139i]. It is excreted mainly unchanged but 6- and 7-hydroxydimethyltryptamine are also excreted, chiefly as glucuronides. Szara and Axelrod[139j] suggested that it could form the following

metabolites: 6- or 7-hydroxydimethyltryptamine, 6- or 7-hydroxyindolacetic acid (HIAA), N-methyltryptamine, tryptamine and 3-indolacetic acid (3-IAA). DMT in street samples can be detected using the TLC technique of Kaistha *et al.*[41].

(ix) Bufotenine. Bufotenine is 3-(2-dimethylaminoethyl)-5-hydroxyindole (also known as 5-hydroxy-N-dimethyltryptamine, N,N-dimethylserotonin and Mappine). It is an indole alkaloid found in some species of *Piptadenia* and *Amanita* and in the secretions of the parotid gland of toads. It is said to have a similar hallucinogenic action to that of DMT. Bufotenine has been reported as a constituent of human urine. Its presence has been demonstrated in the urines of schizophrenic patients[139k]. Bufotenine and its metabolites are excreted mainly in the urine. About 15% is excreted as 5-hydroxyindolacetic acid and the major metabolite is the glucuronide conjugate of bufotenine[139l].

(x) Psilocybin. Psilocybin is 3-(2-dimethylamincethyl) indol-4-yl dihydrogen phosphate. This is the main indole alkaloid present in the mushroom, *Psilocybe mexicana.* It is a hallucinogenic compound having similarity in action to that of LSD and bufotenine. It is rapidly converted in the body by dephosphorylation into psilocin which appears to be the psychoactive compound. About 11% of a dose is excreted in the urine as unchanged psilocin and about 20% as a glucuronic acid derivative. About 3.5% is demethylated and degraded to indole acid derivatives[139m].

(xi) Psilocin. Psilocin is 3-(2-dimethylaminoethyl)-4-hydroxyindole (also called 4-hydroxydimethyltryptamine). It is an indole alkaloid obtained from the mushroom *Psilocybe mexicana.* Psilocin is less stable than psilocybin. It is thought to be an active hallucinogen.

(xii) Phencyclidine (PCP). Phencyclidine [1-(1-phenylcyclohexyl)piperidine] abuse is becoming increasingly common. In 1969 it was marketed under the trade name Sernylan for veterinary anesthesia. It was abandoned for human use in 1965 because of its severe psychomimetic and sympathomimetic actions. It is also known as PCP, angel dust (sprinkled on parsley and smoked), tic and tac, rag, hog, sheets, Hawaiian woodrose, mist, Rocket Fuel, crystal, Crystal joints (CJS), Peace Pill and the Monkey tranquilizer[140,141]. The acute effects of phencyclidine begin at once and subside in about an hour. There is a feeling of sleepiness, a decrease in pain and touch sensation, blurred or double vision, slurred speech and muscle weakness[142]. The effects of phencyclidine and the experience in treating the emergency outpatient cases at the University of Chicago, hospitals and clinics have been discussed in detail by Fauman *et al.*[140,140a]. Phencyclidine is rapidly metabolized to a hydroxylated derivative and excreted in the urine as a piperidine conjugate. The metabolite has little pharmacological activity[142,143]. Aronow *et al.*[144] recently reported their observations that urinary excretion of PCP was markedly pH dependent. Acidification of the urine by administration of ammonium chloride caused a 10-fold increase of clearance in a urine of pH 6.5 and more than 100-fold increase when the urine was 5.5 or lower. Acidification was also associated with clinical improvement. We have been able to detect unchanged phencyclidine in the spinal fluid and urines of overdosed patients by the TLC procedure of Kaistha *et al.*[41]. This life-saving service of urinalysis has been gratefully acknowledged by Fauman *et al.* in their recent report[140a]. The drug is extracted out of the available biological fluid at a pH of 10.1 (ion paper is not used in such cases since only 5–10 ml of fluid is available). However we have extended the

use of ion-exchange paper technique to some of our clients who are alleged to have used phencyclidine provided the urine voided is 20 ml or more.

(xiii) Marihuana (marijuana) and cannabis. The abuse of marihuana in our society is on the increase. The National Institute of Mental Health in its second annual report to Congress[145] estimated the number of marihuana users at between 15 and 20 million. Approximately half of the number were believed to use it one or more times per month. The National Commission on Marihuana and Drug Abuse[145] estimated the number of users to be somewhat higher, at about 24 million. A recent brochure by the Drug Abuse Council[146] pointed out that 6% of the students in the high schools and 8% of the students in the colleges admitted daily use of marihuana. In the U.S.A., marihuana is taken by inhaling the smoke from the cigarettes called "reefers". It is also known by different street names such as tea, Mary Jane, pot, the weed, love weed, Indian hay, joy smoke, locoweed, laughing grass, grass, hashish or hash. Other names for cannabis products include charas, ganja, dagga and bhang. In botanical term the common hemp is an herbaceous annual, of which *Cannabis sativa* is the sole species and *Cannabis indica* and *americana* are some of its varieties. The resinous exudate of the tops of the female plant contains most of the active ingredients; in the Middle East and North Africa the resin is called hashish; in India it is called Charas. In India, the dried leaves and flowering shoots of the female plant which contain smaller amounts of the active substance are called bhang, and the resinous mass from the small leaves and brackets is called Ganja. In the U.S.A., the term marihuana is used to refer to any part of the plant or extract therefrom capable of producing psychological effects. Readers interested in knowing more about the psychological effects and pharmacological actions are advised to refer to *The Pharmacological Basis of Therapeutics*[147] and to Neumeyer and Shagoury[148]. The most active ingredient of cannabis is tetrahydrocannabinol (Δ^9-*trans*-tetrahydrocannabinol or Δ^9-THC). According to monoterpene numbering system, Δ^9-THC is also known as Δ^1-THC, however, the dibenzopyran numbering system has been adopted by National Institute of Mental Health which approves the nomenclature of Δ^9-THC. The metabolism of Δ^9-THC has been studied extensively both in animals and humans[149–164]. A significant study carried out at the National Institute of Mental Health[159] in human volunteers on labelled THC showed that its metabolites appear within 10 min after administration. These workers showed that 30% of the administered radioactivity was excreted in the urine and that less than 1% was unchanged Δ^9-tetrahydrocannabinol. They also found that 11-hydroxytetrahydrocannabinol was one of the metabolites of THC as reported by various research groups[149–164], however, it accounted for only a small percentage of the metabolites. Eighty percent of the metabolites still remained uncharacterized. About 50% of the radioactivity administered was recovered in faeces, of which about 20% was the 11-hydroxytetrahydrocannabinol. Andersen *et al.*[165] reported a TLC method for the detection of the 11-hydroxy-THC in human urine. Kisser[166] reported a TLC method for the detection of hashish components in urine. The urine was acid-hydrolyzed prior to TLC. Just *et al.*[167] reported TLC procedures for the detection of Δ^8- and Δ^9-THC in human saliva. The method involved the use of dansyl chloride to form yellow fluorescent sulfonic acid esters, which were chromatographed and subsequently measured by fluorometry or mass spectrometry (MS). Just *et al.*[168] published another procedure for the detection of Δ^9-THC in human saliva which utilized two-dimensional TLC followed by MS. Kelly and Arnold[169]

reported a GC–MS procedure for the detection of 11-hydroxy-THC and cannabinol (CBN) in human urine. TLC sensitivity of the procedure was 1 ng/ml of urine. The method involved enzymatic hydrolysis of urine; the residue obtained after the evaporation of the organic solvent was silanized prior to GC–MS. A RIA procedure has also been reported by Teale *et al.*[170] for the detection of cannabinoids in blood and urine. The technique is specific for three-ringed cannabinoids without absolute specificity for THC. Since it cross-reacts with 11-hydroxy-THC, the present assay can be employed to detect cannabis use in epidemiological studies, case finding and for clinical purposes by examination of the urine. Recently, Yeager *et al.*[290] reported RIA for chosen metabolites of Δ^9-THC in blood, urine and plasma. A new enzyme-based immunological assay using EMIT has also been proposed by Dubowski *et al.*[291].

H. Miscellaneous analgesics and drugs used in the treatment

a. Meperidine

Meperidine is a phenylpiperidine, ethyl-1-methyl-4-phenylpiperidine-4-carboxylate. It has been marketed under a variety of names including Dolantin, Demerol, Isoripecaine, and Eudolat. Its international non-proprietary name is pethidine. After morphine, it is probably the most widely used and the most effective narcotic analgesic. Meperidine is metabolized chiefly in the liver. In man, it is hydrolyzed to meperidinic acid which appears to be the main route. Meperidinic acid is excreted as free and as well as in bound form. Meperidine is also N-demethylated to non-meperidine, which may then in turn be hydrolyzed to non-meperidinic acid and subsequently conjugated[171]. Very little meperidine is excreted unchanged[172], only 5% of the unchanged form was detected after the administration of 175-mg dose[173]. About one third of administered meperidine can be accounted for in the urine as N-demethylated derivatives. The excretion of meperidine is influenced by the pH of the urine[174], in highly acidic urine the excretion of unchanged drug is significantly increased.

b. Propoxyphene

Propoxyphene has become a widely prescribed drug. Although its efficacy and its toxicity are controversial, it still appears to be an extensively used drug. Cravey *et al.*[175] recently reported the results of a 5-year study from three California counties which showed that propoxyphene was responsible for 238 fatal cases. Propoxyphene is 4-dimethylamino-3-methyl-1,2-diphenyl-2-butanol propionate. The dextro-rotatory salt is marketed as Darvon, the levo-rotatory salt is marketed as Novrad, and its water-insoluble salt, propoxyphene napsylate, is marketed as Darvon-N.

Propoxyphene is readily absorbed after its oral administration. N-Demethylation of the drug in the liver results in the formation of norpropoxyphene which appears to be an important metabolic pathway in both man and animals, only a small fraction of unaltered compound is excreted in the urine. According to Amundson *et al.*[176], the unchanged drug was excreted in the first 6 h after ingestion and the metabolite from 6 to 48 h after ingestion. Approximately 3% of a 130-mg dose appeared unchanged in the urine within 24 h and about 5% in 72 h[177,178]. Propoxyphene can be detected in human urine using TLC[41,179]. Several GC methods have been reported for the determination of propoxyphene in urine, plasma and tissues[180–184]. Thompson *et al.*[185] and Wallace *et al.*[178] proposed the use of spectrophotometry. In a more recent report, Wallace *et al.*[186] utilized a combination of UV spectrophotometric and GC techniques. McBay *et al.*[187] recently reported that most of the propoxy-

phene in liver and urine is conjugated and that acid hydrolysis not only liberates the conjugated form but also causes the formation of a new compound which has an UV absorption far greater than the parent compound. Immunoassay reagents using EMIT are also available for the detection of propoxyphene and norpropoxyphene[33].

c. Methadone

Methadone was first synthesized by the Germans during World War II. Chemically it is *dl*-4,4-diphenyl-6-dimethylamino-3-heptanone hydrochloride. It is known by various names such as dolophine, amidone, physeptone, miadone, butalgin, diadone, adanone, polamidone, and 10820[188]. The analgesic activity of the compound is almost entirely the result of its content of l-methadone. The pharmacological properties of methadone are qualitatively similar to those of morphine. The outstanding property of this drug is its effectiveness as an analgesic (pain killer). The drug also causes sedation, depression of respiration and exhibits effects upon smooth muscle and the cardiovascular system similar to those of morphine[189,292]. Methadone is useful in rehabilitating heroin addicts because it suppresses the withdrawal symptoms and reduces or eliminates narcotic hunger[2]. Methadone undergoes extensive biotransformation, chiefly in the liver; N-demethylation seems to be an important metabolic pathway. It is excreted in the urine and faeces in the form of metabolites, less than 10% being excreted unchanged. A considerable portion of methadone is excreted as metabolites into the intestinal tract by way of the bile[172]. Beckett *et al.*[190] were able to identify metabolites of methadone in man; it was established that methadone is N-demethylated in man to a secondary amine which spontaneously rearranges to a pyrroline derivative. The major metabolite is 2-ethylidine-1,5-dimethyl-3,3-diphenylpyrrolidine; another minor metabolite, characterized by the absence of the second N-methyl group is 2-ethyl-5-methyl-3,3-diphenylpyrroline. Beckett *et al.*[191] reported that normethadone and isomethadone gave corresponding pyrrolines similar to methadone. Sullivan *et al.*[192] were able to detect three additional metabolites of methadone in man and in the rat. It is likely that two of these metabolites are *p*-hydroxy compounds corresponding to the above structures, and both of them were found to be excreted as conjugated (glucuronides or sulfates). The third metabolite was found to be identical with 4-dimethylamino-2,2-diphenylpentanoic acid. Basett and Casarett[193] conducted a quantitative study on the renal elimination of methadone and its major metabolite by subjects on a methadone maintenance program. Excretion of methadone was markedly enhanced by acidification of the urine; while the metabolite was less affected by changes in urine pH. The dependency of methadone excretion on pH of the urine is of considerable importance in a methadone maintenance program. Patients are maintained at a dosage designed to eliminate narcotic hunger while permitting to perform normal daily chores of life. Changes in diet, occupational exposure to other chemicals, ingestion of other drugs, and other factors may alter the pH of the urine thus upsetting the steady state of the established methadone dose regimen which in many cases could be the valid reason for requesting a change of dose by some of the clients. The authors[193] have also reported sex difference in the pattern of excretion; women appear to have higher metabolite–methadone ratios in urine. Methadone in the urines of clients attending methadone maintenance programs can be detected using conventional TLC techniques of Cochin and Daly[194], Dole *et al.*[195,196], Davidow *et al.*[197,198], Mulé *et al.*[199,200], Kaistha and Jaffe[38,40,104] and Kaistha *et al.*[41]. An immunoassay technique using activated lyso-

enzyme (EMIT)[201,202], and a RIA technique using ^{125}I-labeled methadone antigen[34] are also available. Both of these techniques have proved to be specific for monitoring methadone in drug abuse prevention and treatment programs. The specificity of RIA[34] has been recently validated by Manning *et al.*[203]; phenobarbital showed some cross-reactivity at a concentration of 10 μg/ml of urine. Quantitative GC[190,204,205], and spectrophotometric procedures[205–207] have also been reported for the determination of methadone and/or its metabolites in biological specimens.

d. α-l-Acetylmethadol

Levo-α-acetylmethadol is α-6-dimethylamino-4,4-diphenyl-3-acetoxyheptane, more commonly it is known as LAAM or Methadyl Acetate. Its various other names are α-Acetylmethadone, Alphacemethadone, α-Amidone Acetate, α-Methadol Acetate. The drug is effective in relieving narcotic hunger and suppressing withdrawal symptoms for a two- or three-day period in contrast to 24-h relief afforded by methadone. Its clinical evaluation for the treatment of opiate addiction[2,208–210] has shown that, when given three times a week, it is successful in suppressing the abstinence syndrome for the vast majority of patients without significant adverse effects. It is safe and can not be distinguished from methadone by patients or physicians blind to the medication being administered to a particular patient[2]. LAAM can be substituted for methadone and *vice versa*, thus now creating more flexibility in treating clients on methadone maintenance programs. There is little euphoria caused by LAAM because of its slow onset and long duration of action making it less abusable. The patient for the first time on LAAM learns that daily dosage of maintenance medication is not necessary and he or she can function without the pharmacologic crutch of daily high. The metabolism of LAAM has been studied by Way and Adler[172], Billings *et al.*[211–213], Sung and Way[214], Smits and Booher[215], Kaiku and Inturrisi[216,217] and Finkle *et al.*[218]. The drug is disposed of essentially by degradation; N-demethylation is one metabolic pathway. Less than 2% of the drug is excreted unmetabolized[217]. Four metabolites have been reported[216–219]: (i) nor-acetylmethadol or nor-methadyl acetate (6-methylamino-4,4-diphenyl-3-heptanol acetate); (ii) nor,nor-acetyl methadol or nor,nor-methadylacetate or dinor-methadylacetate; (iii) methadol (6-dimethylamino-4,4-diphenyl-3-heptanol) and (iv) nor-methadol (6-methylamino-4,4-diphenyl-3-heptanol). Kochhar *et al.*[220] reported a TLC procedure for the separation of α-acetylmethadol, nor-acetylmethadol, and methadol. They found only two metabolites (methadol and nor-acetylmethadol) in their *in vitro* study on microsomal preparation of rat liver. McIntyre *et al.*[221] described a TLC procedure for the separation of four metabolites in human urine. The four metabolites detected by these authors appeared at various times up to 72 h after ingestion of LAAM. One of the metabolites which appeared in the later stages of metabolism had the same R_F value as that of unchanged drug but it could not be the unchanged LAAM because of its late appearance. This metabolite had also the same R_F value as that of the major metabolite of methadone. However, the metabolite due to LAAM was not reactive to ninhydrin spray but the major metabolite of methadone was amenable to ninhydrin spray after its exposure to UV, which was the only observable difference between LAAM containing and methadone-containing urine at the later stages of metabolism. Kaistha and Jaffe[38] detected the presence of two metabolites in urine using TLC, one of the metabolites was observed at the level of methadone from 6 to 76 h. GC procedures

have also been reported for the detection of *l*-α-acetylmethadol and/or its metabolites in biological specimens[211,216,217,219].

McIntyre *et al.*[221] also tested the LAAM- and methadone-containing urines with EMIT[201,202] and reported that two of its metabolites did react with this activated lysoenzyme system.

e. Pentazocine

Pentazocine is chemically 1,2,3,4,5,6-hexahydro-6,11-dimethyl-3-(3-methyl-2-butenyl)-2,6-methano-3-benzazocin-8-ol and belongs to the benzazocine series (also known as the benzomorphan series). Its brand names are Talwin and Fortral. Pentazocine is a potent analgesic which when administered orally in a 50-mg dose appears equivalent in analgesic effect to 60 mg of codeine. It is an extremely weak morphine antagonist. It also has sedative activity[222]. It is alleged to be widely used by drug-dependent individuals attending methadone maintenance programs. Pentazocine biotransformation in rhesus monkey has been studied more thoroughly and is known to be similar to that in man. Pittman and Portmann recently reported[223] the relative proportions of known metabolites and their conjugates in urine of rhesus monkey. In addition to unchanged pentazocine, the following metabolites were recorded: *trans*-alcohol and its conjugated form; *cis*-alcohol and its conjugated form; and the carboxylic acid metabolite from *trans*-alcohol in the conjugated form. The average recovery of the dose in 24 h in the urine of all human subjects was 61.5% of a dose of 56.5 mg of pentazocine hydrochloride administered orally. A total of 9.5% of the dose was excreted as pentazocine in 24 h; 11.4% of the dose was excreted as the *cis*-alcohol metabolite in 24 h; an average of 40.6% of the dose was excreted as the *trans*-acid metabolite in 24 h[224]. Readers interested in more details about the biotransformation of pentazocine in humans, mice, rats and monkeys are advised to refer to ref. 225. Unchanged pentazocine can be detected in the urines of drug users using the TLC technique of Kaistha and Jaffe[40], and Kaistha *et al.*[41].

f. Cyclazocine, naloxone and naltrexone

Narcotic antagonists are effective against heroin and other narcotics because they prevent these drugs from reaching the nervous system. They differ from methadone, in that they themselves do not have narcotic effects and are not addictive. According to one school of thought the drug-seeking behavior is reinforced positively by self-administration of narcotics and since this group of drugs can block the effects of self-administered narcotics which, it was stipulated, would extinguish the drug-seeking behavior. These drugs have been found to have the capacity to block the effects of administered heroin. In addition, they prevent the development of physiological dependence and afford protection from death by overdose. The usefulness of cyclazocine and naloxone has been assessed in many clinical centers, and their application appears to have limited value[2]. Kurland *et al.*[226] have reported their experience on the use of naloxone as a low-dose maintenance program. One of the problems encountered in assessing the usefulness of these antagonist drugs is their too short duration of action and the fact that they provide no incentive to the subjects to return for frequent maintenance therapy. To overcome these shortcomings, efforts have been made to prepare sparingly soluble salts and salt complexes so that intramuscular injection of such forms could provide slow release of drugs and a useful prolongation of action.

Zinc tannate complexes of cyclazocine, naloxone and naltrexone have been developed[227–229]. Naltrexone, a close analog of naloxone has been reported to be essentially as pure an antagonist as is naloxone and to be two to three times as potent with about twice the duration[229]. Naltrexone zinc tannate has been found to provide a highly promising increase in duration of action in mice. Duration was further enhanced when the complex was incorporated in an aluminum monostearate gel. No information is yet available on the clinical assessment of these complexes in the treatment of heroin users.

Cyclazocine is a benzomorphine compound: 3-(cyclopropylmethyl)-1,2,3,4,5,6-hexahydro-6,11-dimethyl-2,6-methano-3-benzazocin-8-ol. No information is available on its metabolism in man. Naloxone is 1-N-allyl-7,8-dihydro-14-hydroxy-normorphinone (benzomorphine compound). It is also known as Narcan and Narcone and Narcan Neonatal (Endo Labs). Naloxone has been reported to undergo glucuronidation, N-dealkylation, and reduction of the 6-oxo group. It is excreted as 7,8-dihydro-14-hydroxy-normorphinone,N-allyl-7,8-dihydro-14-hydroxynormorphine and naloxone 3-glucuronide in addition to the unchanged molecule[230–232]. Cyclazocine (unchanged) and naloxone (unchanged) can be detected using the TLC technique of Kaistha and Jaffe[40] and Kaistha *et al.*[41]. Kokoski[233] has also reported a TLC technique for the detection of unchanged naloxone in human urine. A GC procedure for the detection of naloxone has been reported by Weinstein *et al.*[234]. Naltrexone (unchanged) can also be detected using the TLC technique of Kaistha *et al.*[41].

4. DEFINITIONS OF COMMONLY USED TERMS IN URINALYSIS

A. Sensitivity

Detection methods available for the identification of drugs of abuse in biological fluids are evaluated on the comparative ability to detect very low concentration of the desired drug and/or its metabolites per milliliter of the fluid. This ability to detect the minimal concentration of the desired constituent is called sensitivity.

A detection procedure must uniformly express the concentration of a drug and/or its metabolites that it can detect as μg/ml or ng/ml of undiluted urine. If a statement says that "the test detects 50 μg", it means "the test has a sensitivity of 50 μg", but please note that this statement does not specify the volume of undiluted urine that originally contained the 50 μg. For statement of sensitivity, it is vitally important to specify the volume of undiluted urine that contained this 50 μg. For example, if this 50 μg were contained in 20 ml of urine, the sensitivity of the detection technique would have been 2.5 μg per ml of undiluted urine; if this 50 μg were contained in 50 ml of urine, the sensitivity of the technique would have been 1 μg/ml of urine; or if this 50 μg was contained in 100 ml of urine, the sensitivity was 0.5 μg per ml of undiluted urine. Sensitivity also means the cut-off limit or the threshold concentration of a drug that a technique can detect. If a published technique has a sensitivity of 0.5 μg per ml of urine of the unchanged drug and/or its metabolite, it means that the result will be negative in a voided urine specimen if the concentration of the unchanged drug and/or its metabolite is less than 0.5 μg per ml of urine and the result will be positive if the concentration is 0.5 μg or more than 0.5 μg per ml of urine.

B. Reproducibility

A reported sensitivity of a technique must be reproducible when repeated several times. If two published techniques have the same sensitivity, one could be superior to the other technique depending on their individual ability to reproduce the results. For example two techniques A and B have the same sensitivity of 100 ng per ml of urine; the conventional way to find out their reproducibility will be to take a certain number of controlled urines, say 10 specimens, add drug to each urine so that the final concentration is 100 ng/ml. Then carry these urines through the published assay procedures. Technique A might be able to identify that drug in 9 of 10 samples while the technique B might be able to identify that drug only in 5 of 10 samples. Thus technique A will be superior to the technique B and will be considered reproducible and reliable.

C. Selectivity and specificity

These terms refer to the ability to determine the desired constituent in the presence of other substances. In the case of urine tests, if a technique can discriminate the desired drug and/or its metabolites from other closely or structurally related drugs and/or their metabolites, and naturally occurring substances, the technique will be considered to be selective and specific.

D. False positive and false negative tests

A false positive test situation is a most serious error since it could be extremely damaging to the individual. If the urine specimen on analysis shows that the client has used a particular drug which in fact he has not, the test is considered to be false positive. Whenever a counselor has a reason to believe that his or her client has not used the reported drug, the result must be challenged and the Laboratory should be asked to validate the result of the reported drug. Since multiple drug use is becoming increasingly common, the opportunity of reporting a false positive result increases especially if the technique used is not specific. A false negative result indicates that the drug is not present when it actually has been used. A technique can produce a false negative result if the voided urine specimen has less concentration of the drug and/or its metabolite than the lower threshold sensitivity or the cut-off limit of the technique. A good test must be able to detect drug use for urines obtained in the first 24 h after ingestion. The time elapsed between urine collection and drug intake is very critical. Another important parameter will be the volume of urine voided and the volume of urine needed for the technique used by a laboratory. A sample of urine voided after 60–72 h of heroin use would be negative and could still technically be called false negative though the technique used could be superior according to above definitions. It is therefore imperative that the counselor be familiar with the metabolic rate of the drug in question and also with the usual technique which his service laboratory employs.

E. Turn-around time

This term refers to the amount of time taken in a laboratory to complete the analysis of one sample. It does not include the time taken to collect the sample and to transport it to the testing laboratory (receiving time) nor the time taken to transmit the result to the counselor who will interpret the result (sending or mailing time). To avoid any delay in transmitting the results, it is good practice to telephone the results to the concerned counselor, simultaneously confirming these results by mailing a written report. Without reference to other factors, the definition of turn-around time, as given above, is not useful since laboratories usually do not process one sample at a time in order to avoid the increase in cost of analysis; rather they process several specimens at a time. A more useful concept would be throughput, *i.e.*, the number of specimens that one technician can analyse in an average time of 7.5–8 h work shift. This would include the time it takes to get the various equipment, walking time to use this equipment, preparation of reagents, perform analysis, and to interpret and/or to record the results. This would also depend on the number of tests performed per specimen. A technique could have a short turn-around time if only one test is performed per specimen and the same technique could have an increased turn-around time if more than one test is performed per specimen, *e.g.*, when using immunoassay techniques.

F. Number of tests

Recently, this term has been used loosely to indicate the number of urine specimens analyzed. Such use of this term in order to indicate the number of urine specimens analyzed (workload) should be discontinued because it fails to differentiate between a technique having the ability to detect only one drug *versus* the technique which has the ability to perform multiple detections at a time per urine specimen. For example an immunoassay procedure which has the capability of performing only one test at a time could be claimed to perform 400–500 tests per day without mentioning the number of specimens actually analyzed. If only one test per urine specimen is performed, only then would this statement of 400–500 tests per day be correct, otherwise the quantity of specimens analyzed per day would decrease substantially from 400–500 to 100–125 specimens per day if four tests per specimen are performed. By the same token, a laboratory using TLC could claim that a technician in their laboratory is performing 400–1200 tests per day (testing 4–12 different drugs per specimen) without mentioning the number of urine specimens actually analyzed per day. Alternatively the same laboratory could say that a technician in its laboratory is analyzing 100 specimens per day without mentioning the number of tests performed per urine specimen. It is therefore, essential that these terms be used in their proper perspective.

5. DETECTION PROCEDURES IN CURRENT USE

Kaistha[235] was the first to discuss in depth the detection procedures which were available for mass screening of drugs of abuse in urine until the year 1972. This was followed by a monograph[236]. Later on, the National Clearing House for Drug Abuse Information published a fact sheet[237] on the methods for the detection of drugs of abuse in body fluids. Techniques to-date can be broadly categorized as follows:

TLC, GLC or GC, liquid chromatography (LC), GC–MS, spectrofluorometry, and immunoassays. These methods vary greatly with respect to their suitability for use in large-scale urine monitoring programs.

A. Criteria for evaluation of techniques

The criteria by which a method should be judged are: (1) effective throughput and rapidity (maximum drug indentification in a minimum amount of time), (2) sensitivity, (3) adaptability for mass screening of specimens, (4) versatility and expansibility (ability to test simultaneously a wide variety of commonly abused drugs and/or their metabolites), (5) specificity, (6) convenience and (7) cost effectiveness.

I have been a strong proponent of using inexpensive, versatile but specific and reliable urine detection procedures for clients attending multimodality drug abuse prevention treatment programs. The demand for urine screening is increasing from such areas as pre-employment screening of job applicants in many industries, evaluation of impaired workers, detection of drug users among criminals and the detection of stimulant drugs in athletes. Urine testing has now been added as a service to rehabilitate drug-related arrestees under the various programs such as "Treatment Alternatives to Street Crime" (TASC), "Work Release Programs" (ex-addicts in prison and on parole) and "Freedom Alternatives, Confrontation, Equality and Success" (FACES) under the department of Correction. In choosing the most appropriate type of urine detection procedure, one would always consider the needs, the peculiar requirements, and the intended use of urine data by each of the above programs. For example, quantitative analysis and the use of a sophisticated technique such as GC–MS are not needed for clients attending multimodality drug abuse prevention treatment programs. In the multimodality situation, a technique of unequivocal qualitative specificity would be needed, the one which could provide an adequate "Yes" or "No" answer for the possible use of various drugs of abuse without any false positive results. This author firmly believes that no client should be discharged or separated from a program solely on the basis of a dirty urine report without concomitant involvement in criminal activities or antisocial behavior. We must keep in mind that the data generated from the urinalysis is intended to be used as a yard stick to measure the efficacy of the treatment modality and to monitor the client's progress.

However, the needs of other programs involving punitive action on the basis of a dirty urine report are slightly different than those of treatment programs and therefore all positive qualitative results in such cases must be confirmed by an alternate technique having a superior specificity. These programs do not need quantitative analysis. Similarly analysis of samples required for emergency medical diagnosis of overdosed patients do not require quantitative work. Medicolegal cases and the cases involving criminal guilt *do* need mandatory, unequivocal, quantitative and qualitative analyses obtained by at least more than two different techniques including GC–MS. Furthermore, drug concentrations in urine samples without blood or plasma analysis could rarely be interpreted to yield any meaning with respect to behavior or clinical condition. The only sure interpretation that can be derived is that the subject has ingested the drug. The time has now come to institute clear-cut distinctions between the needs of a forensic toxicology and those of the treatment programs. Detection

techniques used for urine monitoring in treatment programs do not need quantitative percentage recoveries of various drugs. However these techniques must meet the criteria (1)–(7) as outlined above.

B. Thin-layer chromatography

At present, TLC is the most suitable technique for large-scale screening of urines for drugs of abuse and it meets all the criteria (1) to (7). Additionally, it is simple, needs minimum instrumentation, minimum laboratory space and provides excellent resolution of components. A laboratory personnel with minimal formal training can perform the complete drug abuse urine analysis including interpretation of results. With the TLC method it is possible to detect simultaneously a wide variety of drugs of abuse in a single run. Analysis of a urine specimen by TLC alerts the operator immediately as to the number of drugs present in the specimen. In addition, the method can be easily adapted to the purpose of screening, *e.g.*, screening of a client's urine in a treatment program for specific drugs of abuse, or pre-employment screening for a variety of abused drugs. The TLC method can differentiate illicit drugs and their adulterants from legitimate and prescribed drugs and their metabolites. Recently TLC has been used in conjunction with immunoassays as a means of confirming the positive result reported by an immunoassay. Furthermore, an unequivocal specificity can be achieved by the careful design of the developing solvent and the subsequent selection of the visualization techniques. The results are qualitative and not quantitative, *i.e.*, they provide only "yes/no" or "positive/negative" result. Because of its specificity, effective throughput and cost effectiveness, TLC is often used as the only method of analysis for urine monitoring in treatment programs. The technique can detect reliably most of the drugs of abuse and/or their metabolites in a client's urine at concentrations of 0.1–1 μg per ml of urine.

There is no universally accepted procedure for TLC. Numerous TLC procedures are available which already have been discussed by Kaistha[235]. There are three basic steps which are fundamental to all TLC procedures: (1) pre-chromatographic extraction step or clean-up procedure, (2) separation of drugs on the TLC plate, and (3) the detection and read-out of the separated drugs. It is due to the pre-chromatography extraction step as well as to the variety of detection procedures used that the TLC techniques vary considerably from program to program. In fact, the superiority of a published TLC technique as applied to the detection of drugs in human urine can be attributed to the efficiency of the pre-chromatographic extraction step and the specificity and the sensitivity of the visualization techniques used.

First step. The pre-chromatographic extraction step, also called clean-up procedure or preparation of sample is designed to isolate and concentrate the drugs from the impurities in whole urine. Several different extraction techniques involving three basic approaches have been reported in the literature:

(1) Liquid–liquid extraction of the urine, also called organic solvent extraction or direct extraction of drugs from a urine specimen at various pH's;

(2) extraction of drugs from urine by absorbing them on a resin column ionic or non-ionic such as XAD-2 resin at a controlled pH and then eluting with organic solvents or absorbing the drugs and/or their metabolites on a cation-exchange resin loaded paper and then eluting them with different solvent systems;

(3) acid or enzymatic hydrolysis of urine specimens followed by direct extraction of drugs. These three basic approaches for the extraction of drugs have already been discussed elsewhere[235].

Numerous liquid–liquid extraction procedures for the extraction of various drugs of abuse from urine and other biological fluids have been reported in the literature[40,104,179,194,197–200,238–242]. However, the single-step direct extraction procedure of Davidow *et al.*[197,198] is the one which is widely used by various drug abuse urine monitoring laboratories.

Marks and Fry[243] used an ion-exchange resin column for the extraction of morphine from urine specimens. Fujimoto and Wang[244], and Quame[245] were the first to use non-ionic Amberlite XAD-2 resin columns for the separation of narcotic analgesics and other drugs of abuse. These columns later on were placed on the market by the Eastman-Kodak and Brinkmann Instruments[246]. Several procedures to increase the efficiency of these columns have been published by Miller *et al.*[247], Kullberg *et al.*[248], Mulé *et al.*[249], and Bastos *et al.*[250,251]. Kullberg and Gorodetzky[252] were able to increase the recovery of drugs up to 75–93% by eluting various drugs from the column with acetone and methanol–chloroform. The urine was buffered to pH 8.5 prior to passing through the resin column. Three methods of recovering morphine from morphine glucuronide were also investigated using these resin columns. Hydrolysis of urine followed by resin extraction of the liberated morphine was proved to be superior to the other two methods. It must, however, be pointed out that the efforts to increase the recoveries of various drugs using more polar solvents such as methanol or various other combinations of ethylene dichloride–ethylacetate or ethyl acetate followed by methanol would result in removing absorbed urine pigments and other naturally occurring urine contaminants from the resin. These contaminants would not only result in dirty residues but also could be the source of false positives.

Dole *et al.*[195] were first to suggest the use of Reeve Angel SA-2 cation-exchange resin-loaded paper to absorb the drugs from urine. Barbiturates, opiates and amphetamines were eluted from the paper with 3 consecutive extractions at pH 2.2, 9.3, and 11, respectively. Jaffe and Kirkpatrick[253] proposed a two-step extraction procedure for the elution of barbiturates at pH 2.2 and opiates and amphetamines at pH 9.5. Heaton and Blumberg[254] modified the procedure reported by Dole *et al.* and extracted narcotics, amphetamines, and psychotropic drug metabolites from cation-exchange paper at pH 9.3–9.4. They reported that the procedure of Dole *et al.*[195] yielded poor recoveries for barbiturates and amphetamines. Mulé[199] modified the procedure of Dole *et al.*[195] and recommended the use of 50 ml of undiluted urine. Using this modified technique, he still reported poor recoveries for barbiturates, methadone, and amphetamine. Kaistha and Jaffe[40,104,242] reported a modification of the method developed by Dole *et al.*[195]. They eluted sedative hypnotics at pH 1 and opiates and amphetamines at pH 10.1 using NH_4Cl–NH_4OH buffer. Using this modification, they were able to detect barbiturates (except sodium barbital), amphetamine, methamphetamine, phenmetrazine, and opiates including methadone at a level of 0.5–1 μg per ml of urine. Gorodetzky[255] modified the procedure of Dole *et al.*[195] for the extraction of morphine. He utilized two pieces of 6 × 6 cm cation-exchange resin-loaded paper instead of one and the urine containing these resin papers was shaken for 60 min. He obtained 48.2% recovery of morphine as compared to the

modified organic solvent extraction procedure (liquid–liquid extraction). The organic solvent extraction procedure of Mulé[199] was also modified by him and the recovery of morphine obtained was about 60–60.4%. These efficiency studies on the modified ion-exchange paper technique were conducted by adding concentrations of 0.3 to 3.6 μg of morphine per ml of urine. Kaistha *et al.* in their recent communications[41,256] reported single-step extraction of opiates, amphetamines, barbiturates and a wide variety of other abused drugs from the cation-exchange resin-loaded paper. The sensitivities obtained for the various drugs of abuse were superior or equal to the organic solvent extraction procedures or XAD resin column techniques. The volume of urine required for these sensitivities was 20–50 ml. Kaistha and Tadrus recently reported[257] that minimum shaking of 20–30 min is essential for the maximum absorption of drugs by the cation-exchange resin-loaded paper. The same results could be obtained by soaking the ion-exchange resin-loaded paper overnight with intermittent shaking. These investigations also revealed that the concentrations of various drugs added to the urine to establish the sensitivity of the cation-exchange resin-loaded paper were too high, *i.e.*, the ion paper does not need 0.3 μg of morphine per ml of urine to get a positive reading but the concentration of 0.1 to 0.2 μg of morphine per ml were found to be almost quantitatively absorbed by the ion paper. It was on the basis of these results that a sensitivity level of morphine was established between 0.1 and 0.19 μg per ml of urine. Two more modifications to improve the efficacy of the ion-exchange paper technique have been reported by Kaistha and Tadrus[32,258]. These modifications consisted of increasing the extraction (shaking) time to 20 min and changing the chloroform–isopropanol ratio.

The conclusion is justified that the use of ion-exchange paper technique presents more advantages over the organic solvent extraction and/or XAD-2 resin column techniques. For convenience and control, the adsorption of drugs on the paper can be done in a clinic or field station and the paper (with the patient's name, date, and relevant clinical data written on it with a lead pencil) rather than having the liquid urine specimen sent to the laboratory. It is far simpler and markedly less expensive to transport ion papers than either raw urine or resin columns through Postal or United Parcel Service from remotely located treatment units to a centralized laboratory. The risk of contracting any viral or other infection through the processing of raw urines by the laboratory personnel is virtually eliminated by the use of ion paper. In addition to simplicity and convenience, the use of ion-exchange paper has an intrinsic economy which encourages its use in keeping a continuous vigil over the drug using activities of a client. A counselor can, as his needs require, collect multiple urine drops from a single client and then place all related urine drops in one plastic bag for a single shipment to the laboratory followed by a single analysis when it arrives. The entire week can thus be covered without increasing the cost of analysis.

The next step, *i.e.*, evaporation of the organic solvent is common to all of the above pre-chromatographic extraction procedures. Solvent evaporation is generally performed by addition of acid to the solvent to convert heat-labile free-base drugs such as amphetamines to their stable salts.

Another aspect of sample preparation is the hydrolysis step. Since as much as 83% of total morphine[114] and 88% of the total codeine[259] may be excreted as their glucuronides, acid or enzymatic hydrolysis of urine specimens collected infrequently is of great value. Acid or enzymatic hydrolysis converts water-soluble conjugates of

morphine and codeine into free bases. The usual methods of sample preparation do not extract these water-soluble conjugates. It is, therefore, apparent that the addition of a hydrolysis step will further enhance the ability of chromatographic techniques to detect these drugs. Various procedures currently used to hydrolyze urine specimens were discussed by Kaistha elsewhere[235].

Second step. The second basic step in chromatography is separation of the drugs. This step first involves the spotting of the residue on a plate precoated with a thin layer of solid support phase, usually silica gel. In TLC, the transfer of the residue from the test tube on to the plate is the most critical step. In fact the state of the art of TLC lies in quantitative spotting of the residue. The plate is then placed in a developing solvent and by capillary action the solvent slowly moves upward on the plate in a uniform manner. The solvent is allowed to rise to a certain distance to the extent the resolution of the components is desired. The plate is then taken out and allowed to air dry. The separation of various drugs and their metabolites is achieved because different drugs migrate or travel different distances from the starting point. It must be pointed out that the efficacy of a TLC technique primarily depends on the proper design of the developing solvent, a poorly designed developing solvent such as too polar solvent mixture could result in false positives for various drugs of abuse. Various solvents currently used in drug abuse urine monitoring laboratories were discussed by Kaistha in detail elsewhere[32,40,41,235,258].

Third step. The final step involves the detection of the separated drugs and their interpretation. Detection of various drugs and their metabolites is achieved by spraying the plate with chemicals which produce characteristic colored spots with various drugs. By the combination of specific spraying reagents and the use of UV light, different drugs can be specifically identified. Detection procedures currently used for the specific detection of various drugs of abuse were discussed earlier[32,40,41,235,258].

C. Gas chromatography or gas–liquid chromatography

GC is essentially a technique of separating a mixture into its components. This technique is used in conjunction with TLC to verify the findings made by TLC. The sensitivity of this technique is almost the same as that of TLC but can be enhanced by the use of electron capture or nitrogen detectors. It has the inherent problem that only one specimen at a time can be monitored per detector and it is time consuming. However, like TLC, it permits simultaneous screening for a variety of drugs. Using GLC, a single specimen may require 20–30 min for the complete screening of opiates and amphetamines. Although its specificity is claimed to be superior to TLC, different drugs and their metabolites can have similar retention times. The use of this technique alone without TLC is not advised.

GLC involves the same three basic steps as in TLC: (1) sample preparation or pre-chromatographic extraction step, (2) separation of drugs, and (3) detection. The separation step in GLC differs from TLC in that the sample is injected into a gas chromatograph and volatilized (liquid converted to gas). The components, in the gaseous state, are forced through the column by a carrier gas. The column usually has a small diameter and is loosely packed with an inert solid support coated with a stationary phase (liquid phase). The principle of separation is based on the partitioning

of the drug between the stationary phase and gaseous phase. Thus a mixture of compounds is separated into individual components which reach the end of the column and then enter the detector through the detector lines at different times. The time taken for each component to pass through the gas chromatograph is different and is characteristic for a particular drug and is called retention time. The detector at the end of the column detects the presence of the compound and makes a graphic presentation on the recorder in the form of a peak. The graph also indicates the retention time of the drug. Identification is based on comparing the retention time of the unknown compound with a known standard.

Scientists and medical technologists working in the field of toxicology, forensic chemistry, and drug abuse detection are advised to refer to the chapter on GC by Leach[260], *Handbook of Analytical Toxicology* by Sunshine[261] and a paper by McMartin and Street[262]. The applications of GLC in drug abuse urine screening programs were discussed by Kaistha[235] earlier.

D. Liquid chromatography

High-speed LC is the newest rapidly developing method of separating thermally labile materials, polar and highly water-soluble drug metabolites in biological fluids and confiscated street drugs[263,264]. It can be used both for qualitative and quantitative purposes. The technique is of great value to the forensic toxicologist confronted with a wide variety of complex analyses involving biological fluids[265]. LC is a simple form of instrumentation based on three elements: solvent delivery system, injector and column, and detector. A major difference between GC and high speed LC is the pumping system required to deliver mobile phase through the column at a reasonable flow-rate. This technique can not yet be used in large-scale urine screening programs due to lack of rapidity and simplicity.

E. Gas chromatography–mass spectrometry

The combined use of GC–MS technique is becoming exceedingly important in the area of forensic toxicology. This technique offers the best available answer to the forensic needs of getting unequivocal identification of drugs and their metabolites extracted from biological fluids. It is direct, fast, sensitive to minute concentrations. The instrument employs a single-column GC for separation of drug extracts from biological samples. The separated components are automatically transferred to a quadrupole-type mass spectrometer for analysis. The principle is as follows. Molecules from the gas chromatograph enter the ion source where they are bombarded with electrons which are emitted from a hot filament. The neutral molecules are ionized to form a variety of products, including positive ions. The positive ions are used in the analysis. While ionization can occur at any bond in the molecule, it does not occur at certain preferred locations giving rise to a distribution of ions which constitutes a finger print of the original molecule. The positive ions are electrically extracted from the ion source and injected into the quadrupole mass filter where they are separated according to their mass. The ions passing through the quadrupole filter are quantitatively detected by an electron multiplier, amplified and the resulting signal is fed into an appropriate display. This system is known as conventional electron impact (EI)

MS. The spectra produced by this system are quite complex, materials introduced must be in a relatively pure state. This requirement is usually accomplished by the separation of a material's components on a gas chromatograph. The recent development of a new MS ionization process known as chemical ionization (CI) has made the mass spectrometer a versatile instrument. A combined EI–CI source provides the analyst with the option of identifying multicomponent mixtures without prior chromatographic separation. A wealth of literature is now available on the applications of GC–MS to forensic toxicology. A comprehensive GC–MS reference data has been generated by Finkle *et al.*[266,267]. Readers are also advised to refer to the technical bulletins issued by the Finnigan Corporation and Hewlett-Packard[268,269]. This technique is currently being used in street drug analysis for the unequivocal identification of some drugs not identifiable by TLC and GLC. It has no applications in mass screening of urines in treatment programs due to lack of simplicity and rapidity. Furthermore, it is too expensive.

F. Spectral methods

Spectrophotometric techniques can be subdivided into following categories: (a) UV and colorimetric spectrophotometry, (b) infrared spectrometry, (c) atomic absorption spectrometry, (d) mass spectrometry, and (e) spectrofluorometry. All of these techniques have wide-spread use in pharamaceutical research and analysis as well as in the broad area of organic and inorganic analysis. They also provide valuable information on the structural identification and elucidation of unknown molecules. Their usefulness for the identification and quantitative analysis of various drugs of abuse and their metabolites was discussed earlier by Kaistha[235]. However, most of these techniques are not widely used in large-scale drug abuse screening programs because they lack simplicity and rapidity and are too expensive. Only fluorometry, also called spectrophotofluorometry, has been used in drug abuse urine screening programs.

a. Spectrophotofluorometry (SPF). The applications of this technique for the qualitative and quantitative identification of drugs of abuse in biological fluids were discussed by Kaistha[235]. The fluorescence process is characterized by two spectra. A fluorescent molecule emits its fluorescence spectrum after it has absorbed radiation anywhere within its excitation spectrum. The spectral distribution of the fluorescence radiation is a physical and absolute characteristic of a given substance and is useful for qualitative information. The emission intensity of fluorescence at a given length is useful for quantitative analysis. The principle of this method is based on the fact that many drug derivatives known as drug fluorophores, emit fluorescent light under specific conditions. The beam of light at a certain wavelength is passed through the sample which causes the fluorophore to emit light at another specific wavelength. The technique involves preparation of the sample or a clean-up procedure before the extracted drug can be converted into a fluorophore by a specific reaction. There are two well demonstrated methods[270–272] for the production of morphine fluorophores: one uses potassium ferroferricyanide to generate fluorescent pseudo morphine, and the other depends upon sulfuric acid oxidation. There are two commercial instruments presently available which apply this methodology. Farrand (Mount Vernon, N.Y., U.S.A.) markets an automatic turret SPF instrument which permits semi-automation, and Technicon (Tarrytown, N.Y., U.S.A.) has developed a fully automated SPF system

for morphine. However, Technicon has withdrawn its system from the market but it can be purchased if desired.

G. Immunoassays

During the past five years potentially useful immunoassay techniques such as the free radical assay technique (FRAT)[273,274], EMIT[273,275], RIA[276,277], hemagglutination inhibition (HI)[278,279], and the latex flocculation test (LFT)[280], applicable to drug abuse screening programs, have been developed. The applications of these techniques to the analysis of morphine, related surrogates, and other drugs of abuse have added a new dimension to the detection and/or identification of these drugs in biological fluids and tissues. These tests meet some of the criteria such as relative simplicity, rapidity, high sensitivity and adaptability to high volume for their selection as drug abuse urine monitoring techniques. These techniques do not require preliminary sample processes such as extraction or hydrolysis. However, they lack the criteria of effective throughput and expansibility, *i.e.*, the simultaneous detection of a wide variety of drugs of abuse in minimum amount of time, which are the requirements needed due to the current trends of polydrug or multiple drug ingestion and the shifts in abuse pattern from one drug to another drug. Furthermore, they are less specific and prohibitive in cost. One of the main advantages of the immunoassays is high sensitivity. Compared to TLC and GLC, specificity is not good and is the main disadvantage. Sensitivity and specificity seem to be interrelated with immunoassays, *i.e.*, the assays which are very sensitive are less specific and *vice versa*. Their high sensitivity enabled detection of heroin use for a longer time as recently reported by Gorodetzky[18] and Gorodetzky *et al.*[281]. Their usefulness lies in reporting a negative result as a strong presumption of the absence of the drug at the time the urine is voided. However, a negative result does not mean that the drug was not used for several days, it could be safely interpreted that the drug was not used for the last 40–72 h. According to Gorodetzky[18], EMIT could detect 38% of urines positive up to 40 h and RIA could detect 39% of urines positive for total morphine up to 72 h after a single i.v. dose of heroin at a concentration of 10 mg/kg. It must be pointed out that all positive tests obtained by these techniques must be confirmed by an alternate non-immunological technique of high specificity such as TLC or GLC. The authors of the monograph[236] *A Guide to Urine Testing for Drugs of Abuse* and the fact sheet[237] by National Clearing House stated that TLC has the sensitivity of 0.5–1.0 μg and 1.0–3 μg per ml of urine for morphine and other drugs. However Kaistha and co-workers[8,32,41,256–258] have reported that free morphine can be detected at a level of 0.1–0.2 μg per ml of urine using TLC. In fact we have found that a technician of average capability in our quality control system in which we introduce 4% of blind samples having 0.17–0.19 μg of morphine base per ml of urine can routinely report positive results with an accuracy of 98% or more. A well trained technician has the capability of detecting morphine at the concentration of 0.1 μg per ml of urine. We also introduce blind controls for other drugs of abuse at the sensitivities reported in our publications.

Mulé *et al.*[282] recently published the results of their evaluation of immunoassay techniques and reported that all the immunoassays were reliable within the limitations of assay and the number of false positives ranged from 3 to 31%. However, the TLC concentrations used by these authors for comparison were 1–2 μg for morphine

and 3–5 μg for amphetamine per ml of urine which we believe were in excess of the levels of concentrations routinely required in day to day laboratory operations. Readers interested in more information on immunoassay techniques are advised to refer to the review article by Mulé[283] and ref. 284. Cross-reactivities (lack of specificity) of various immunoassay procedures were discussed by Kaistha and Tadrus[8], Mulé[283], and Finkle[285].

6. DEVELOPMENT COSTS OF TOXICOLOGY LABORATORY AND COST PER URINE TEST

Kaistha and Jaffe[286] reported the initial cost of setting up a toxicology laboratory facility in a drug abuse urine screening program using TLC method. Kaistha[235] later on discussed the comparative costs of TLC using ion-exchange resin-loaded paper and XAD-2 resin columns, GLC and FRAT. With the advent of numerous immunoassay techniques, a necessity was felt to assess the cost of each available detection procedure on the basis of its capacity to perform the number of tests per specimen and number of specimens per day including reagents, ancillary supplies and labor cost. Readers interested in knowing these and other details pertaining to TLC, GLC, SPF (Farrand semi-automated spectrophotometer and Technicon automated system), EMIT, FRAT, RIA, HI and LFT are advised to refer to ref. 8. A summary of this data is given in Table I for ready reference. It may be pointed out that our current total unit cost of analysis using TLC including supervisory and administrative salaries (one chief toxicologist, one laboratory manager, one chief chemist), chemicals and supplies, laboratory rental and overhead charges, technical and support services, is about $1.38 per specimen for monitoring 3800–4200 specimens per week[32,258].

7. PROFICIENCY TEST SURVEYS

The U.S. Center for Disease Control (CDC), Department of Natural Health and Welfare, initiated nation-wide proficiency testing surveys about 5 years ago. The purpose of instituting these surveys was to evaluate the existing detection techniques employed by various testing laboratories and to upgrade their testing proficiency. Ten urine specimens are shipped to each participant every three months. Drugs currently used in these surveys are morphine, codeine, barbiturates, amphetamine and/or methamphetamine, methadone and its major metabolite, cocaine and/or its metabolites, propoxyphene and benzodiazepines. The matrix of these samples are human urines, some are addict's urines and some urines are spiked with the above drugs and/or their metabolites. Although five years have passed, the concentration of drugs used are still very high. Last year only in one survey the concentration of free morphine was lowered to 0.25 μg/ml, however, the concentration of morphine-3-glucuronide added was still high, *i.e.*, 1.7 μg/ml. All the testing laboratories must be able to report the lower concentrations for each drug if these laboratories have to provide excellent service to their clients. The following concentrations for each drug and/or their metabolites are considered most desirable for laboratories involved in drug abuse screening:

free morphine, 0.1 μg per ml of urine (this should be the ultimate goal), the total concentration in a urine specimen including morphine-3-glucuronide should not exceed 0.5 μg/ml;

codeine, 0.5 μg/ml;

barbiturates, 1 μg/ml;
amphetamine and methamphetamine, 1 μg/ml;
methadone and/or its metabolite, 1 μg/ml;
cocaine, 1–1.5 μg/ml; benzoyl ecgonine, 2 μg/ml;
propoxyphene and norpropoxyphene, 1 μg/ml;
benzodiazepines; these drugs are not excreted unchanged in appreciable amounts so their metabolites should be used, with a concentration of 1–2 μg/ml.

The various combinations of drugs added should be able to generate meaningful information about the validity of the various detection techniques used. Codeine

TABLE 1

SUMMARY OF COST PER URINE SPECIMEN (Sp)

M = morphine, C = codeine, Q = quinine, Me = methadone, A = amphetamine, Mpt = methamphetamine, P = phenmetrazine, Mpn = methylphenidate, B = barbiturates, Be = benzoylecgonine (cocaine metabolite).

Technique	*Start-up cost*	*Total delivery (Sp/day) and cost per Sp (on the basis of 1 test per Sp)*	*Total delivery (Sp/day) and cost per Sp (on the basis of ⩾4 tests per Sp)*
TLC	*ca.* US$4,400 including equipment, expendables and chemicals	135 (4 tests/Sp) US$0.58 per Sp	90 (9–14 tests/Sp) US$0.82 per Sp 135 (4 tests/Sp) US$0.58 per Sp
GLC	US$4,000–8,000, plus US$500 (ancillary supplies)	45 (>4 drugs/Sp) US$0.88	25 (opiates, amphetamines and barbiturates) US$1.48 per Sp per 5–7 tests
SPF			
Farrand system	*ca.* US$6,000 (but instrument can be leased or rented)	400 US$1.09 per Sp per test	200 *ca.* US$2.0 per Sp per 2 tests (M and Q)
Technicon system	US$25,000 (but instrument can be leased or rented)	300 US$0.22–0.29 per Sp per test	150 *ca.* US$0.44 per Sp per 2 tests (M and Me)
EMIT	*ca.* US$7,100, semi-automated plus US$200 for miscellaneous supplies[8]	450–500 US$0.60 per Sp per test[8]	112–125 US$2.40 per Sp per 4 tests
FRAT	US$26,000	400 US$0.71–1.57 per Sp per test[8]	100 US$2.84 per Sp per 4 tests
RIA	US$9,000 for gamma counter plus ⩽$500 for centrifuge	625 US$1.26 per Sp per test[8]	150 US$4.64 per Sp per 4 tests
HI	US$400–500 for centrifuge, titer trays and Pasteur pipets	300–400 US$0.51 per Sp per test	100 *ca.* US$2.0 per Sp per 4 tests
LFT	*ca.* US$500	300–400 (commercially not available as yet)	(not available)

* Refs. 32, 41, 256–258.
** Equally sensitive to codeine.
*** Specificity comments on morphine are not listed for EMIT, FRAT, RIA, HI and LFT since all immunoassays can not differentiate heroin use from legitimate codeine use and other structurally related narcotics.

should invariably be included in at least one specimen because it is a widely prescribed drug and a service laboratory should be able to differentiate codeine from heroin use. Mixtures of amphetamine and methamphetamine should be avoided since a laboratory may not be able to detect methamphetamine. For example, when a mixture of these two drugs is used, laboratories using only RIA technique would still be able to record a positive result for amphetamine. Similarily many laboratories do not have the capability of testing separately amphetamine or methamphetamine at the concentration of 1 μg/ml. However, an immunoassay technique not able to detect amphetamine at a concentration of 1 μg/ml will report a positive result due to the additive effect of methamphetamine. Phenylpropanolamine, a commonly used

Sensitivity (μg/ml)										*Specificity (at sensitivities stated)*
M	*C*	*Q*	*Me*	*A*	*Mpt*	*P*	*Mpn*	*B*	*Be*	
0.1–0.2*	0.5*	0.5*	0.5*	1.0*	0.5*	0.5*	1.0*	0.4–0.5*	0.5–2.0*	Very good to good for all drugs; inter-laboratory variation depends on developing solvents and detection reagents (steps 2 and 3). A practical level of 0.2 μg free morphine per ml of urine is suggested for all laboratories.
0.1–0.5[236]	—	—	—	1–2[236]	—	—	—	1–2[236]	0.5–1	Very good to good.
0.22[288]	—	—	—	—	—	—	—	—	—	Very good[236].
0.20	—	—	—	—	—	—	—	—	—	Good[236].
0.3	**	—	0.3	1–2	1–2	—	—	1–2	1	Good for Me and Be, moderate for A, Mpt and B***
0.1–0.5	**	—	0.5	1.0	1.0	—	—	1	1	The same as EMIT for drugs other than morphine***.
0.025–0.1	**	—	0.1	1	—	—	—	1	0.1	Good for A; can not test Mpt; good for Me, Be and B***.
0.025–0.50	**	—	—	—	—	—	—	—	—	***
0.1–0.2	**	—	—	—	—	—	—	—	—	***

decongestant should also not be mixed with amphetamine or methamphetamine since a laboratory otherwise not capable to differentiate phenylpropanolamine from amphetamine or methamphetamine will still report a positive result from amphetamines. TLC techniques used by certain laboratories and some immunoassay techniques can not differentiate phenylpropanolamine from amphetamine or methamphetamine. Therefore it is important that urine specimens spiked with phenylpropanolamine do not contain amphetamine or methamphetamine. Similarily mixture of barbiturates should also be avoided since a mixture of phenobarbital and secobarbital each at a concentration of 1 μg/ml will increase the capability of certain immunoassay techniques due to the additive effect. A technique which may be unable to detect phenobarbital at a concentration of 1 μg/ml will still report a positive result if secobarbital has also been added at a concentration of 1 μg/ml. The mixture of cocaine, propoxyphene or the mixture of methadone and cocaine are highly desirable to see the efficacy of the TLC techniques used by various laboratories.

The concentration of benzoyl ecgonine currently used is too high, it should be lowered to 2 μg/ml. Necessary steps to include phenmetrazine (Preludin) and if possible methylphenidate should be taken. The inclusion of phenmetrazine is quite important since it is alleged to be widely used.

8. SOME QUESTIONS AND ANSWERS ON DAY-TO-DAY PROBLEMS

Why is quinine tested in the urine, although it does not belong to opiates class?

It is tested because in certain geographical regions, it is extensively used to dilute (cutting agent) street heroin. Its presence in the urine is a very good indicator of a street drug usage.

What steps should be taken by a counselor to use quinine as a tool for street drug usage?

A counselor should advise his clients not to use any quinine water with hard liquors. No client should ingest any on-the-counter preparation containing quinine without the prior knowledge of his or her counselor.

What other drugs can give a positive quinine test?

In addition to quinine water and quinine preparations, bromo-quinine and quinidine sulfate can give a positive quinine test. Quinidine as sulfate or gluconate is used for the management of certain cardiac arrhythmias and is a prescription drug.

What other adulterants are used to cut heroin in addition to quinine?

Antihistamines such as methapyrilene (Histadyl), and procaine (Novocaine) a local anesthetic are two other adulterants commonly employed to dilute heroin.

What adulterant is used to cut cocaine?

Procaine (Novocaine) is a common adulterant used to dilute cocaine.

Can methylphenidate (Ritalin) be used to clear or block the appearance of morphine and quinine from the system prior to voiding the urine specimen?

No. Ingestion of methylphenidate (Ritalin) will not affect the clearance of mor-

phine or quinine from the body. The testing laboratory should be able to test for morphine and/or quinine without any foreseeable problem.

Can ingestion of diuretics and/or large quantities of fluids eliminate the appearance of morphine in urine?

Ingestion of diuretics and/or large quantities of fluids will increase the total output of urine, and thus can dilute the concentration of morphine in a voided urine specimen. The time course of morphine and/or its metabolites to eliminate completely out of the system may change to a certain degree as compared to a normal individual not ingesting any diuretics or large quantities of fluids.

Are there any drugs which will clear the urine from morphine without showing themselves in the urine?

No. There is no drug known to the author which could eliminate the appearance of morphine in a urine specimen. However a urine report generated by a laboratory may not show the presence of other drugs in the urine, because drugs other than listed on a particular report are not recorded.

Is a mistake made at the clinic level as damaging to the situation as an error in the laboratory?

Yes, very definitely, such as switching of a urine specimen. A strict control is suggested at the time the urine specimen is voided, also necessary steps should be taken by a counselor to see that urine specimens are not switched during the storage time in the clinic.

Does the ingestion of vinegar by a client after heroin intake eliminate the presence of morphine in the urine? Does this ingestion of vinegar interfere with the laboratory test for morphine?

No. Current rumors on the use of vinegar to eliminate the presence of morphine in the urine are not valid. Urines from a client who used to ingest vinegar were supplied to this laboratory by one of our programs. All urines showed strong positive morphine.

How frequently should the urines of a client be collected to break the drug-seeking habit or to keep continuous vigilance over drug-using activities?

This decision would vary from individual to individual. In initial stages, at least for the first six months of stay in a treatment modality, urine frequency should be spaced on the basis of 3 drops a week such as on Monday, Wednesday, and Friday. Negative urine results for morphine and other drugs of abuse for a period of 4–6 months will be a very good indicator of decreasing the frequency of urine drops to twice a week and then to once a week on random basis. Individuals who are treated for polydrug use are advised to get once a week analysis for the entire array of drugs of abuse such as morphine, codeine, amphetamine, methamphetamine, phenmetrazine (Preludin), methylphenidate (Ritalin), barbiturates, and tranquilizers such as chlorpromazine (Thorazine) and trifluoperzine (stelazine). Drugs such as diazepam (Valium) and flurazepam (Dalmane), cocaine are advised to be analyzed on special requests as the testing of these drugs involves the testing of their metabolites which have to carried out by a testing laboratory separately.

Which are the drugs where the simultaneous ingestion of alcohol should be avoided by a client?

Clients should be advised to avoid the use of alcohol in the case of commonly prescribed antihistamines or their preparations such as actifed, benadryl, chlortrimeton, (chlorpheniramine), histadyl (methapyrilene); and in the case of Darvon, codeine and its products, Donnatal, Elavil, Equanil, Fiorinal, Librium, Librax, Lomotil, Ornade Capsules, Talwin, Stelazine, Thorazine, Tofranil, Tuss-Ornade capsules, and Valium. The drugs listed above may cause drowsiness and alcohol may intensify this effect. Clients should use care when operating a car or dangerous machinery. Alcohol should not be taken with following drugs: Chloral hydrate, Dalmane, Flagyl, Griseofulvin, Nembutal, Noludar, Orinase, Placidyl, Quaalude, Seconal and Tuinal.

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10. SUMMARY

Urine screening for determining illicit drug use has become a necessary adjunct to treatment of drug dependent individuals. The results provide the physician with his only objective measure of progress in treatment. This guide discusses in depth the following information pertaining to urine testing: (i) why urine testing is necessary for clients attending multimodality treatment programs and what should be the frequency of urine collection; (ii) biotransformation of drugs in the body and interpretation of urinalysis data; (iii) definitions of commonly used terms in urinalysis; (iv) overview of currently used detection procedures pertaining to abused drugs and drugs used in the treatment; (v) development costs of a toxicology laboratory facility and cost per urine test; (vi) proficiency testing; and (vii) some questions and answers on day-to-day problems. The author hopes that physicians, clinicians, therapists, program directors, and drug counselors, involved in the day-to-day management and counseling of drug dependent individuals will be able to make better clinical interpretations of urinalysis data in more effectively combating the drug abuse problem. This guide should enable drug administrators and/or executive and clinical directors to make meaningful decision in the choice of an appropriate toxicology laboratory facility, the types of drugs to be tested by urinalysis, the treatment monitoring efficacy and the continuing evaluation of the need for additional treatment.

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