13th Annual Meeting

October 11 - 14, 1983

Book Cadillac Hotel
Detroit, Michigan

Society of Forensic Toxicologists

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HEROIN DISTRIBUTION IN MATERNAL AND POSTNATAL BODY FLUIDS, CNS AND TISSUES OF RHESUS MONKEYS

L. Roizin, J.C. Liu, D. Pierson, W. Rivers and J.A. Meyers, Dept. of Neuropathology and Neurotoxicology and Dept. of Comparative Medical Sciences, N.Y.S. Psychiatric Institute and Dept. of Pathology, Columbia University, N.Y.C.

The concentration of morphine (Coumbis) and Kaul's, modified radioimmunoassay technique) in various body fluids (urine, bile, milk and vitreous humor), CNS (cerebral cortex, basal ganglia, brain stem, cerebellum and spinal cord), organs (lungs, heart, liver, kidney, stomach, intestine, pancreas, spleen and uterus), and body tissues (adrenals, fat, muscle, testis and thymus) was comparatively higher in both mother and offspring treated for longer period of time. After discontinuation of heroin administration, while morphine concentration decreased or disappeared from most of the body fluids and tissues, it remained in significant amounts in the bile, vitreous humor and, at times, in certain regions of the CNS. Our studies demonstrate that the Rhesus Monkey is a very useful experimental model for the study of heroin effects, distribution and treatment; and the differential morphine concentration and retention (after discontinuation of administration) in certain body fluids and tissues may be of particular significance in forensic investigations.

Supported, in part, by NIH Grant #5301DAO-2225.

A TRIAD OF SELF-DESTRUCTION

Alstott, R.L., Lee, D.T., Waeckerlin, R.W. & Rugotzke, G.G., Public Health Laboratory, 517 Hathaway Bldg., Cheyenne, WY 82002 and Natrona County Memorial Hospital, Dept. of Pathology, Casper, WY 82601.

Three cases will be presented. The first describes a suicidal ingestion of malathion by a cancer patient who was currently undergoing radiation and chemotherapy. Malathion concentrations were 5.0 and 18.3 mg/L in blood and urine respectively. No other drugs were found.

The second case is that of a nonfatal ingestion of LSD. Large amounts were found to be present in gastric contents. Patient history suggested an encephalopathy but microbiological tests were negative. Blood and urine were later submitted for examination.

The third case involves suicidal ingestion of a large amount of salicylates by a 73 year old white female. The ingestion occurred under unusual circumstances. Blood and vitreous salicylates were 60 and 35 mg/dl respectively. Tissues were also analyzed.

FATAL CASES INVOLVING SOME COMMON ANTI-DEPRESSANT DRUGS

D. Semple, S. LaDelpha, B. Joynt, D. Ballantyne, Royal Canadian Mounted Police, Central Forensic Laboratory, Ottawa, Ontario, Canada.

The involvement of many new psychoactive drugs in cases of accidental overdose or suicidal death is frequently noticed soon after their introduction. A report on the occurrence of maprotiline, amoxapine and a new experimental drug zimelidine in various cases encountered in the Central Forensic Laboratory, Ottawa will be described. Analytical data for GC, MS and TLC will be presented, as well as quantification procedures. The blood/tissue concentrations for these drugs ranged from therapeutic to fatal.

PREDICTION OF ION-PAIR PARTITIONING CHARACTERISTICS OF SOME TOXICOLOGIC ANALYTES

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The partitioning characteristics of a series of basic analytes of toxicologic interest and their ion-pairs were determined. The two sets of partition constants were linearly related in the low pH range and appear to be independent of the pKa values of the individual analytes. Distribution diagrams of the ion pairs and the free analytes provide a basis for prediction of selective extraction and separation.

APPLICATION OF REGULAR SOLUTION THEORY IN THIN LAYER CHROMATOGRAPHY.

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With careful attention to technique highly reproducible ($\sigma<0.01$) thin layer chromatographic (TLC) R_f values of a series of analytes of toxicologic interest were obtained in a series of solvent blends. Using the measured R_f values in and the calculated Hildebrand cohesive energy density (δ) values of the solvent blends the solubility parameters of the analytes were determined. From the slopes of plots of R_f against a function of δ's the molar volumes of the analytes were calculated. The cohesive energy density and molar volume values may be used to predict TLC behavior of analytes in other solvent blends. This report describes development of the method and its application to analytes of forensic interest.

SOLUBILITY PARAMETER VALUES AND MOLAR VOLUMES OF POLYCYCLIC DRUGS: HIGH PRESSURE LIQUID CHROMATOGRAPHIC DETERMINATION.

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Highly reproducible ($\sigma<0.01$) high pressure liquid chromatographic (HPLC) retention volumes (V_r) of a series of polycyclic drugs of toxciologic interest were determined in a series of solvent blends. Using the measured V_r values in and the calculated Hildebrand cohesive energy density (δ) values of the solvent blends the solubility parameters of the drugs were determined. From the slopes of the plots of V_r against a function of δ's the molar volumes of the drugs were calculated. The cohesive energy density and molar volume values may be used to predict HPLC behavior of the drugs in other solvent blends. This report describes development of the method and its application to drugs of forensic interest.

PROBLEMS IN THE POSTMORTEM DIAGNOSIS OF ACUTE CYANIDE POISONING.
Bryan Ballantyne, Applied Toxicology Department, Union Carbide Corporation, Charleston, West Virginia.

Autopsy findings are few and nonspecific in acute lethal cyanide poisoning. The cyanide odor may be missed because of masking or since the pathologist is unable to detect the smell. Postmortem production of cyanide in tissues, giving a false positive result, is unlikely. However, when normal blood is stored under deep freeze conditions cyanide is produced, but not to levels likely to be of forensic significance. Postmortem transformation of cyanide occurs in intact bodies or stored tissues, and could result in a false negative diagnosis. There is rapid transformation of cyanide in blood in intact bodies, but this is significantly less in blood stored after sampling. The tissues most appropriate for analysis following poisoning by any route of exposure are brain, myocardium and spleen. In cases that have been treated in hospital before death, it requires to be remembered that certain antidotal drugs may interfere with analysis for cyanide. Decreased cytochrome oxidase activity of diagnostic significance can be dtected in brain and myocardium, but not if measurements are delayed for several days after death.

THE EFFECTS OF 2,3-DIPHOSPHOGLYCERATE ON THE PREPARATION AND STABILITY OF BLOOD CARBON MONOXIDE. Raymond J. Bath Ph.D., Janet Weiswander, University of Illinois College of Medicine at Rockford, 1601 Parkview Ave., Rockford, Illinois 61107-1897

2,3-Diphosphoglycerate (DPG) occupies the cavity of the hemoglobin molecule in the deoxygenated state. As oxygen or carbon monoxide is "bound" to the hemoglobin molecule rotation of the protein changes the cavity size and DPG is eliminated from the cell. At death changes in oxygen content and pH can change the DPG equilibrium. Thus forensic analysis of just bound carboxyhemoglobin can be misinterpreted. The preparation and stability of standard carboxyhemoglobin blood without careful analysis of DPG content can lead to erroneous values.

"Stability of Basic Drugs in Frozen and Formalin Fixed Tissue over a One Year Period"
B.R. Hepler, R. Solano, D. Rosenhamer, C. Sutheimer and I. Sunshine, Cuyahoga County Coroner's Office, Cleveland, OH
Abstract:

Equivalent amounts of liver, brain, kidney and spleen obtained post mortem from selected cases where amitriptyline, nortriptyline, imipramine, desipramine, pentazocine and triplennamine had been ingested were stored frozen at -20°C or fixed at room temperature, in 0.5L of formalin solution. At six month intervals, 6 gram aliquots of each tissue were homogenized and, following a double extraction clean-up into a final small volume extract of dichloroethane, assayed on OV-17 gas chromatographically using a FID. Additionally, the formalin solutions bathing the fixed tissues were assayed for drug content at 1,3,4,26 and 52 week intervals. Within run and between run precision for the liver tissue assay varied from 4-7% and 7-19% respectively. Qualitative and quantitative (following normalization of the data) results over the period studied demonstrated that, (i) storage at -20°C is optimum for consistancy and clarity of results, (ii) evaluation of the formalin bath can be qualitatively useful and, (iii) further work is necessary in the standardization of tissue sampling techniques.

PRELIMINARY STUDIES OF THIORIDAZINE-5-SULFOXIDE CARDIOTOXICITY IN THE ISOLATED, PERFUSED RAT HEART. Paul W. Hale, Jr. and Alphonse Poklis, Depts. of Pharmacology and Pathology, St. Louis University School of Medicine, 1402 S. Grand, St. Louis, MO 63104

The cardiotoxicity of a racemic mixture of thioridazine-5-sulfoxide, an oxidative metabolite of thioridazine, was studied in the isolated, perfused rat heart. Thioridazine-5-sulfoxide (ring sulfoxide) was prepared by a new method which allows for separation of the 2 stereoisomeric forms of the compound. Hearts from male Spraque-Dawley rats were perfused using a modified Langendorf preparation. Quinidine (6.2×10^{-5}M) served as a positive control. Perfusate concentrations of the ring sulfoxide as low as 1.2×10^{-5}M produced premature ventricular contractions, bradyarrythmias, and aberrant ventricular conductions. Arrhythmias progressed from first degree block to complete AV block. Effects were reversed by removal of the drug, within 90 minutes ECG's were normal, but heart rate returned to only 80% of control. These findings suggest a cardiotoxic potential for the ring sulfoxide.

DEATH BY ARSENIC: A COMPARATIVE EVALUATION OF EXHUMED BODY TISSUES IN THE PRESENCE OF EXTERNAL CONTAMINATION
Joerg N. Pirl, G. Fred Townsend, Ausrine K. Valaitis,* Dietmar Grohlich, John J. Spikes
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External contamination by inorganic arsenic compounds of hair or nails cannot effectively be removed with H_2O or dilute HCl. The use of a modified Gutzeit apparatus allows investigators to establish or rule out external contamination with arsenic compounds. It was found that externally contaminated samples will generate large amounts of arsine gas when treated with Zn, Kl, $SnCl_2$, and dilute HCl in a Gutzeit arsine generator. Arsenic present in the sample due to metabolic deposition resulted in significant arsine formation only after digestion. In one chronic arsenic poisoning case significant amounts of arsine were liberated from nail only after digestion. Direct examination of hair and nail from a female resulted in values near 1000 µg As/g even after washing with HCl and H_2O. Deep thigh muscle and spinal cord, however, released arsine only after digestion. Control hair soaked in sodium arsenate or arsenite solutions, washed repeatedly, and dried, showed maximal binding at 2 hours for $NaAsO_2$, an average of 775 µg of As was irreversibly bound to the exterior surfaces of each gram of hair. An additional 130 µg/g was released upon digestion.

HAZARDOUS WASTES AND THE FORENSIC TOXICOLOGIST

Mark M. Luckens, Ph. D., Lexington, KY 40503

Occupational and societal wastes have reached critical proportions on a global scale. Proper management of civilization's products is a new multidisciplinary profession requiring a knowledge of ecology, engineering, chemistry, geology, environmental and public health, and toxicology.

Forensic toxicology, the performance and interpretation of toxicologic analyses in a judicial context, has a crucial role to play in wastes management as it affects individuals and populations. Wastes introduced into air, soil, and water react with these media and/or their contents, often forming compounds which may adversely affect life forms therein or may enter and contaminate the food chain. Degradation products of apparently innocuous materials may be toxic. Human exposure to these products may elicit an acute, chronic, or lethal response evident in the short term or possibly delayed for years. It is important that the toxicologist be aware of the possibility of a victim's unreported exposure to toxic wastes, effluvia, or their reaction products in deciding on analytic procedures and in interpreting the data therefrom. Failure to consider the role of hazardous wastes and their effects can result in misdiagnoses or serious miscarriages of justice or equity.

DUAL CAPILLARY COLUMN GAS CHROMATOGRAPHIC ANALYSIS OF HYPNOTICS, SEDATIVES, AND TRANQUILIZERS USING DUAL NPD DETECTORS

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Michael J. Ounphy
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Serum is extracted with methyl-t-butyl ether at pH 9 using P-methyl phenobarbital as the internal standard. The extract is evaporated to dryness and reconstituted with methyl-t-butyl ether containing barbital, and injected onto a Dual Column Capillary GC with NP detectors. GC analysis is performed on DB-5 and DB-1701 columns. (J & W Scientific, Inc., 3871 Security Park Drive, Rancho Cordova, CA). The remaining extract is reconstituted in mobile phase (0.05 M NaH_2PO_4 - pH 4- and CH_3CN 65:35) and injected onto C_8 column (IBM Instruments, Inc., 475 Main St, P.O. Box 3020, Wallingford, CT). A separate extraction at pH 9 without internal standard is spotted on TLC plate and visualized for barbiturates, benzodiazepines, glutethimide, and meprobamate. EMIT^R data on serum is also obtained for toxic levels of benzodiazepines and barbiturates.

AN AUTOMATED DRUG SCREENING PROTOCOL BASED UPON HIGH RESOLUTION GAS CHROMATOGRAPHY/MASS SPECTROSCOPY AND RETENTION INDICES

R.R. FREEMAN, HEWLETT-PACKARD CO., AVONDALE, PA

An automated drug screening protocol which utilizes two fused silica open tubular columns and two different detectors: flame ionization and mass selective will be described. From one channel very precise retention data is obtained and retention indices are calculated. From the second channel mass spectra are obtained. A BASIC program correlates the data from the two channels.

A number of toxicology laboratories have developed screening protocols based upon fused silica column gas chromatography. These columns are characterized by a high level of inertness, durability (chemical, mechanical, and thermal), and efficiency which makes them ideal for the rapid analysis of underivatized drugs. In addition, and most important for this application, the column-to-column reproducibility is outstanding.

This method is based upon a simultaneous injection on two "identical" fused silica capillary columns. The columns are installed in the same injection port. One chromatogram is obtained from a flame ionization detector. A retention index is calculated for those peaks exceeding a user-specified threshold. This retention index is then compared to a listing of retention indices.
Tentative identification is based upon an index "match" with those in the library. Quantiation can be via an internal or external standard method.

A second chromatogram is obtained from a mass selective detector. This provides qualitative information about each constituent in the sample. If a drug is "identified" by its' retention index, The mass spectrum (spectra) within a retention time window is(are) compared with those in a "drug" library. Positive identification is based upon the mass spectrum.

This automated method generates both quantitative and qualitative information simultaneously. The identification is unambiguous and sample throughput is maximized. The presentation will focus upon those factors which influence the accuracy and precision of the method.

FORENSIC TOXICOLOGY SURVEY - 1983

H. Horton McCurdy
Division of Forensic Sciences, Atlanta, Georgia

A survey was made of 38 laboratories throughout the United States performing forensic toxicology services. The results of that survey will be presented in regard to case load, services offered, proficiency testing, personnel, salaries, budget and instrumentation.

51-100 ng/mL; c) 101-200 ng/mL; d) 201-300 ng/mL and e) greater than 300 ng/mL. Concentrations of codeine in blood were also determined for all of these categories. Codeine was found to be present in almost all of the deaths (93%). Concentrations of free morphine in blood ranged from 3 ng/mL (cut off point) to 1600 ng/mL. In those cases where free codeine concentrations were determined to be greater than 500 ng/mL, concentrations of free morphine were found to be 1/10 that of codeine, (i.e.) 19/24 (79%) of the cases. Five out of twenty four (21%) were greater than 10% in concentration. Morphine was found in all but one of the above cases. In all but 4 cases was codeine not found when morphine was greater than 51 ng/mL. This study involved 114 opiate deaths.

A RAPID, SENSITIVE METHOD FOR THE SEMI-QUANTITATIVE DETERMINATION OF DRUGS OF ABUSE.

Mark M. Luckens, Ph.D., Lexington, KY 40503.

In 1969, the author described a simple thin layer chromatographic method for the diagnosis of poisoning by organochlorine insecticides. Over the years, this procedure has been modified and used as a screening procedure for the identification and semi-quantitation of drugs of abuse in concentrations at microgram or submicrogram levels per millilitre in body fluids, tissue extracts, dosage forms, powders, or impregnated materials depending on the analyte sought. When indicated, mixtures can be easily separated in advance by two-dimensional or "thick layer" column chromatography. Differences in R_f's, color of the spots, or fluorescence in UV light may be used as modes of separation after visualization. Quantitation may be achieved directly from the spot or in the confirmatory step. The spots may be eluted and the eluate used for confirmation and/or quantitation by GLC, UV, or other procedures.

The equipment may be made from materials readily available in any laboratory or obtained at little or no cost. The liquid and solid phases and visualizing agents, as well as specialized apparatus, are common in forensic laboratories.

Data derived from this method will be compared with those derived from a well-known commercial screening procedure.

CUTTING COSTS OF EMIT^R DAU ASSAYS THROUGH USE OF A PERSONAL COMPUTER

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Replacement of the Syva CP-5000 by an inexpensive personal (micro) computer with interfacing to the Gilford Stasar III and Syva Auto Carousel^{TM} will be discussed. Examples will include the SWTPC 6800 and Commodore 64, although any computer with 17 input and 2 output lines can be used. Some of the advantages of personal computer use are convenient sample identification (Auto Carousel^{TM}), flagging of positive DAU results, decreased transcription errors, and lower purchase price. Typical printouts, programs, and cable diagrams will be shown.

Additional savings through use of a commercially modified flow cell will also be discussed.

PERSISTENCE OF POLYBROMINATED BIPHENYL (PBB) IN HUMAN POST-MORTEM TISSUE.

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PBB, a commercial fire retardant and structurally similar to PCB, was accidentally mixed in livestock feed 10 years ago in Michigan. This accident led to extensive contamination of beef, poultry, pork, dairy products and ultimately to humans. In order to determine the distribution and persistence of PBB, an animal model (rat) was used. The results indicated PBB distribution in all tissues studied and the conclusion that PBB would never be eliminated during the lifetime of the rat.

A second study was conducted to evaluate these findings in humans. 198 post-mortem tissue samples were obtained from 15 subjects who had a history of residency in the Grand Rapids, MI area at the time of the original contamination. The ranges (ng/gm tissue) of the five highest PBB containing tissues were: renal fat, 32-1650; adrenal, 17-868; thymus, 29-617; aorta, 6-1011; and pancreas, 4-653. These results clearly demonstrate long term PBB persistence in the human body and body-wide PBB distribution into tissues other than fat.

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