BEHS 183

Prof. SHULGIN

UNIVERSITY OF CALIFORNIA, Berkeley
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FORENSIC TOXICOLOGY

University of California
BEHS 183
Thursday 12-2 PM

Fall, 1991

Alexander T. Shulgin

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A few years ago, I wrote out every lecture a week ahead of time, and distributed it in advance of the actual class presentation. Then about three years ago I gathered all of these together into this reader. I have just finished going through it in preparation for this year, as there were several things that were poorly explained, or omitted entirely. I hope that each year this will become a little more effective.

It is obviously impossible for you to read the contents of these first two lectures ahead of time, but they are included in this entire set, which is available as a reader selection at Kinko's #1, at 2431 Durant Avenue, which is located in the narrow alley parallel to, and south of Telegraph Avenue, between Durant and Bancroft. Please get these notes, and read them before each following pairs of lectures, so that I will not need to spend time repeating them, but can tidy up things that are not clear. And, what is most important, I can flush out the edges and corners of the topics to be covered, and go off on tangents which your questions and comments might inspire. Copies of the course contents, the list of useful books, and the "Drug-definition Quiz" will be made at the first lecture, but they are reproduced in this reader set.

Lecture #1 is to be an introduction to the course. The name that is given in the catalog for this course is "Forensic Toxicology" but be prepared to have to sit through some 30 or so lectures that will deal marginally, at best, with this subject. We will come back to it periodically as a reference point but from here I hope we will wander off into some new area of interest. A major theme that will again and again recur, will be the philosophy of learning. And, of course, the fun of learning.

Do you have a good foundation in chemistry? This particular subject I consider to be one of the most valuable disciplines that can be brought to the scientific method. It is one of the few disciplines that is incontestably reproducable and explicit. It is usually taught in the sophomore year as a maddening collection of name reactions which are to be memorized and regurgitated on demand. I have often seen the survivors of this trauma being able to give an example of an SN-2 displacement but not being able to guess the physical properties of the product. That is a sad waste. There will be a great deal of chemistry called upon in this course, but only for the single reason that it is often the only exacting discipline which will allow the description of a drug, a process, or an analysis, and there may well be no other starting point.

Do you have a good foundation in biochemistry and the pre- medical disciplines? This is probably the second most important background to have. The study of toxicology is the overlapping of two sciences — the toxin (or the chemical involved) — and the animal (wherein the chemical expresses itself). One must become familiar with the way into the body, the way around the body, and the way out of the body, to ever hope to analyze the body for the presence of a toxin. And the study of the processes into, around and out, will serve as a fine forum for some anatomical review. Here one must consider the magic and chaos of metabolic transformations. Often one must pursue not the toxin itself but rather what the toxin has become or what the toxin has left behind.

Do you have some exposure to instrumentation and to analytical procedures? The "how-to" is of course the heart of a toxicology procedure. Under normal conditions, there is a laboratory section that would accompany this course, and it under normal circumstances it would give modest compliment to this aspect. This year, however, the entire Life Science Building structure is being renovated, so the laboratory section will not even be presented. However, the roles played by the various instruments will be gone into here, as deeply as practical.

My own background is largely chemistry and pharmacology, and I will continuously call upon the vocabulary of these disciplines in my lectures. As will become apparent, I am a hand-waving talker who pays little respect to the lecture plan of the day. That is exactly why I had originally hoped to have these handouts available a week ahead of time, and now they are available at the beginning of the semester for the whole year. Also, I despise memorization. Thus, all exams are open book exams. Bring your notes openly, and bring any reference texts that you or your neighbor might need. And if I can help with some information during the exam, I will make it available to everyone. Someone once described knowledge as that which you have left when you have forgotten everything you've learned.

There will be a handout describing good reference books Become familiar with the names as you might need them some day.

I will try at giving some definitions. Forensic is pretty straight forward. Things dealing with the forum, in other words, open or public. This has come to mean nowadays dealing with the law and the legal processes, but in my philosophy this still means open and public. There is only one goal ever to be sought in forensic anything, and that is the truth. You are a biased person, just as I am a biased person, and any finding that either of us might make will reflect that bias. Your bias may be to please the boss, to knock the Seventh-day Adventists, to promote sex education in high-school, God knows what, but you will have the best control of honesty if you acknowledge these personal biases, and find some way of being at peace with them. The blood on the skirt of that sweet girl that some S.O.B. raped and murdered was not the blood type of the accused. That's my expert opinion. Sorry, Mr. District Attorney.

The definition of Toxicology has already been given. Things to do with drugs. And Pharmacology is the science (knowing, logos) of drugs (drugs, pharmacos). OK. So what is a drug? One of the handouts is a short quiz asking you to give opinions as to which, if any or all, items on a list are, in your opinion, drugs. I want to get a consensus of the definition of drugs from the people I am to be talking to for the rest of the year.

Next week I will return this quiz, and share the results for comment.

One of the major hopes that I have for the year, is to get three or four concepts across. We will touch on dozens of concepts during the semester, but I have a couple of pet ones that are my personal goals for each year. This means not to learn them,

not to understand them, not to play them back on demand, but to really get that Ah Hah gestalt, that light- bulb that appears over the head. Hey, I GET it. Each year I think I succeed, and each year I am disappointed in that many in the class never really Ah Hah'd. Anyone who REALLY gets these will get an excellent grade, regardless of how many wrong answers appear elsewhere on the final. And for those who don't get these – well – it will tell me that next year I must try harder.

What are these concepts? As an example, what is the difference between the chemical basis for atomic weights and the physical basis for atomic weights. Here, I mean the deep, the mandatory, the absolutely inescapable reasons for the two scales. Without this being totally a part of your psyche, you will live in perennial uncertainty in and around the mass spectrograph.

Or the concept that the things that are good about radioimmune assays are the same things that are bad about radioimmune assays. The power of the antigen-antibody interaction and the cross-reactivity that comes with it, is that drugs that are different but closely related to the parent drug can respond indistinguishedly from the parent drug itself, but perhaps to a different degree. The weakness is that drugs that are different but closely related to the parent drug can respond indistinguishedly from the parent drug itself, but perhaps to a different degree.

Or the fundamental difference between solubility and partition. The concept of the partition coefficient of a compound between two solvents has absolutely nothing to do with solubility of that compound in those two solvents. I will hit on this again and again and again with dozens of different approaches and different explanations. One of them might do the trick.

Or the concept of strong inference, coupled with the absolute personal conviction that proof positive is not to be ever realized in any process that is acceptable by science. Here there must be a touch of the fair witness concept of Heinlein's "Stranger in a Strange Land." Never accept an order from your boss to answer the question, "What drug is present in this blood sample?" Only accept the question: "Is heroin present in this blood sample?" The first question may never be answered in a lifetime of diligent dedication. And never answer that second question with, "I have proved heroin to be present." but rather "The presence of heroin is consistent with all of my analyses." Always remember, one contradiction and you have proved it was not there. THAT can be proved. The presence is the consequence of repeated trials to disprove, all of which failed.

And there is, of course, the structure of the exams and the final. I do not like giving grades, but the authorities say, give grades. The idea of pass or fail is marvelous, and those who appear to truly get these several concepts will pass, the others, well, whatever. So I will ask questions which may very well not even have known answers, certainly not known by me. Hence, no right or wrong. But how do you go about working out an answer? What is the thought process that moves your hand? Do I like the way you think? Why is cocaine perchlorate soluble in chloroform, whereas cocaine hydrochloride is not? Damned if I know. Maybe I will get some insight

from your answers.

One last word on the open-book exam topic. The secret is that the answers to the questions will not be in the notes, but in your head. I had a student maybe ten years ago, a medical student from Harvard who was spending a year here on the West Coast to take a breather from his real academic disciplines. His lecture notes were a collection of tape cassettes, and his exam preparation was the thorough indexing of these recorded notes. For the final he brought his Sony, a set of ear-phones, a fast forward index and several sheets of key-words for rapid searching. The first questions dealt with the procedure of making an antibody to LSD in a goat for an RIA analysis. I had never mentioned either LSD or goats during the year. I don't think he ever got to the second question.

YOUR PERSONAL DEFINITION OF THE WORD "DRUG"

A sentence, in its simplest form, has a doer (the subject) which does things (the verb) to something (the object). Using this format, answer the following (selecting from the suggested fill-in words if you want, or choose others according to your own views):

A DRUG IS A _____ WHICH _____ TO _____.
(subject) (does something) (to something)

concept	assists	living things
material	modifies	parts of living things
natural thing	restricts	cells of living things
chemical	changes	behavior
medicine	kills	normal action
anything	affects	

CIRCLE ANY OF THE FOLLOWING THAT YOU WOULD CLASSIFY AS DRUGS:

Propoxyphene	(Darvon, an analgesic)
Ex Lax	(Phenophthalein, a laxative)
Saccharin	(an unmetabolised sweetening agent)
Wine	(a beverage containing alcohol)
LSD	(a scheduled hallucinogen)
Placebo	(a sugar pill without presumed action)
KCl	(potassium chloride, hypokalemia)
Cigarettes	(a nicotine-containing plant)
Marijuana	(a THC-containing plant)
Insulin	(a hormone secreted by the pancreas)
Heroin	(a narcotic synthesized from morphine)
Chicken Soup	(a folk treatment for the common cold)
Cayenne peppers	(capsaicin, a blistering agent)
X-rays	(process to visualize bones)
Vitamin C	(a cure for the common cold?)
Cholesterol	(a food component involved in artery health)

_____ (enter something that you feel IS a drug but which might have been overlooked by someone else in this survey)

_____ (enter something you feel IS NOT a drug, but which some one else may have included in this survey)

USEFUL BOOKS FOR REFERENCE, AND FOR YOUR PERSONAL LIBRARIES

Merck Index 11th ed, 1989; 10th ed. 1983; 9th ed. 1976; all similar to 8th ed. 1968 (several printings)
6th ed. 1952 (organic name reactions)
5th ed. 1940 (some 5000 color reactions

USAN and the USP Dictionary of Drug Names
Published annually, includes mixtures

Physician's Desk Reference (PDR)
Also annual. Medical Economics Co. Has excellent color plates, but beware! All information supplied by the manufacturer.

The Pharmacological Basis of Therapeutics
Goodman and Gilman. The Macmillan Co. New editions every few years. The expert's book.

Cutting's Handbook of Pharmacology
Appleton Century Crofts. Now at about the 6th edition. Compact, concise, accurate and cheap reference gem.

Isolation and Identification of Drugs.
E.G.C.Clarke. The Pharmaceutical Press, London. All English nomenclature but a good start in finding the toxic literature.

Handbook of Analytical Toxicology
Irving Sunshine. The Chemical Rubber Co., poorly indexed, hard to use, and only fair accuracy.

Medicinal Chemistry. Alfred Burger. Now 4th Edition, Wiley Interscience.
An exhaustive encyclopedia of drug chemistry, but now far too expensive.

On-line Data Bases: Current Contents, MedLine. GratefulMed, Chem. Abstracts

PERIODIC PUBLICATIONS

Microgram. Monthly, from the DEA, restricted circulation.
Federal Register. Daily, from the GPO. The true mouthpiece of Government.
Current Contents. Weekly, provides titles, key words and author's addresses.
Citation Index. Far too expensive, but allows you to search forward in time.
Scientific Journals. Analytical Chemistry
Journal of Analytical Toxicology
J. Forensic Sciences (there are two)
Clinical Toxicology

HEALTH STATE OF AN INDIVIDUAL

	ILL (a patient)	WELL (a subject)
The drug is supportive (organizing)	SUPPRESS SYMPTOMS	MAINTAIN STATE
The drug is disruptive (disorganizing)	ELIMINATE SYMPTOMS	GENERATE SYMPTOMS

As examples:

Suppressing symptoms. This represents a cosmetic approach to an illness. You supply the individual with what is needed to overcome the problems of illness, but there is no move towards repair. A person has diabetes mellitus because his pancreas is inadequate, so he takes insulin. This does not help the pancreas and so does not even start to cure the disease.

Eliminating symptoms. This is the direction of cure, and there must be disruption by a drug to achieve this. It must kill a bacterium, or irreversibly bind to a poison, or destroy the reproductive capacity of a cancerous cell.

Maintaining the healthy state. Drugs in this category are primarily for the prevention of disease. Inoculations, antibodies against tetanus and polio, the providing of the body with its eventually needed defenses. And if pregnancy can be considered a temporary pathology (the rapid growth internally, of a body that is not of your unique chromosome make-up, and inevitably fatal if not rejected at some critical point) then contraceptives fit into this category.

Generating symptoms. Certainly, there is a large body of drugs with the purpose to make a well person "sick." In the more accepted areas, there are drugs such as the anesthetics used in surgery You change from an awake, conscious person to a comatose, unresponsive person. And there are the less kosher areas. The use of mind-altering drugs such as the psychedelics should qualify for this category.

The FDA definition of a drug:

(1) Any substance recognized in an official pharmacopoeia or formulary;

(2) Any substance intended for the use in the diagnosis, cure, mitigation, treatment or prevention of disease;

(3) A substance other than food intended to affect the structure or function of the body;

(4) A substance intended for use as a component of a medicine but not a device, or a component, part, or accessory of a device.

Inductive Inference, ex. Francis Bacon:

a. State a hypothesis or hypotheses;

b. Devise an experiment which will destroy one or more of these hypotheses;

c. Carry the experiment through to a clear result;

d. Return to a if you succeed, or to b. if you fail.

And, remember always, it is constructive to ask:

What experiment could disprove this hypothesis?
What hypothesis will this experiment disprove?

PRESCRIPTION DRUGS:

There is a large body of drugs that are assignable to you only by a physician. Some of these are "take-home" drugs which can be given to you by him, or prescribed for you to purchase at a pharmacy. Some of these are only administerable to you at his discretion such as in emergency situations or in hospital treatment. Some of these require multiple prescription forms to be made out if they are legally thought to be especially dangerous. The schedule II narcotics fall in this class. But all prescription drugs can be characterized as having had the approval of the Food and Drug Administration (FDA) for human administration, but having the restriction that only a physician (or in limited cases, another medically trained professional) may make them available. And doctors may always be called upon to justify their uses of prescription drugs to the federal government.

The story of the FDA and prescription drugs has an interesting history (the history of the "narcotic" laws will be covered in Lecture #26). The very first federal drug law was the Pure Food and Drug Act, enacted in 1906. It was written to prohibit "false

and misleading" labels. A 1910 Supreme Court decision proclaimed that this phrase could only apply to the contents of the package – and that usefulness should be judged by the consumer. The Food, Drug and Cosmetics Act of 1938 required manufacturers to provide directions for use and proof of safety. The Bureau of Chemistry in the Department of Agriculture was asked to enforce this law, and it eventually became the Food and Drug Administration, which came to interpret the law to require that certain drugs could be available only by prescription. The entire prescription phenomena was originally a regulation that some drugs were of sufficient risk that the public needed a doctor's advice for their safe use. This was made explicit by an amendment passed in 1951 which codified the distinction between prescription and non-prescription drugs, and gave the FDA sole power to decide which drugs could be sold only on prescription.

Further restrictions came. In 1969 the FDA arranged that the National Academy of Science should conduct a Drug Efficacy Study, and this resulted in a 1970 regulation that "adequate and well-controlled" studies, rather than simple clinical experience, would be required to permit drug availability.

Note, that much that is currently enforceable as law is in fact administrative regulation that has been allowed by law. The original intent of the law has often lost with time.

NON-PRESCRIPTION DRUGS:

All other drugs fall into this classification.

Foreign prescriptional drugs:

These are medically valid drugs that are not recognized in this country. Many drugs are brought back to this country by tourists from Europe or by patients from clinics in Tijuana, that are not recognized in any way in this country. Such drugs, as long as they are not explicitly listed in the Controlled Substances Act (narcotics law violation) or are exchanged in the pretense of the practice of medicine (public health law violation) are without legal definition.

Over-the-counter Drugs (OTC):

This is by far the biggest volume of drug distribution. Here are the things from A to Z that can be bought by consumer choice or by pharmacist recommendation in the drug store, the super-market, the service station, or from High Times magazine. Caffeine (in pills or colas), nicotine, alcohol, or phenylpropanolamine. Many of the scheduled drugs had their abuse potential discovered by the exploration of the OTC inventory.

Black market drugs:

Here fall all the drugs from the illegal (marijuana, heroin, speed) to the not illegal

(look-alikes, pea-shooters, "legal speed") to the out and out fraudulent (misrepresented drugs and synthetic variants sold as something familiar to the buyer).

MARGINALIA:

And there are substances that may very well not be drugs at all. There are spices, health foods, special diets, home remedies, special decoctions (things prepared by boiling), concoctions (things fused together with heat), extracts (things removed by solvents), tinctures (extracts put back into alcohol), and no end of folk-lore medicine. Some of it is valid, and some of it is not.

A most recent palaver brings this drug/not drug argument into sharp focus. There is a plant called Plantain, because of the binomial name *Plantoga psyllium*. It has a soluble fiber that appears to reduce cholesterol. The big company, Procter and Gamble, got the FDA to approve their selling it as the drug Metamucil. Then, just a few days ago, General Mills began incorporating it as a simple grain food into thier breakfast food "Benefit." And they wrote on the breakfast food package, "Reduces Cholesterol." P&G claims foul, that it is a drug and stop making medical claims and get an IND from the FDA. And GM says it is packaged as a cereal, and it looks like a cereal, and it is sold as a cereal. It is a food, so get lost. Is Psyllium a drug or not a drug?

In Scot law, a guilty conviction depends on evidence from two separate sources. If there is acceptable evidence from only from a single source, it is hard to find the accused "not guilty," but the presumption of innocence is tarnished by the fact that there has been some evidence of guilt. So "innocent" seems a little extreme, too. The Scottish third judgment that is found in these cases can be "not proven" and this judgment equally well applies to the validity of many of the folk-lore medicines of the world.

Forensic Toxicology
BEHS 183 Lect. #3

NATURAL POISONS
Some definitions:

POISON:

Any substance which may cause damage to the structure, or disturb the function, of the body. It doesn't really matter whether inserted in, applied to, or developed within the living organism, there is the implication of destructiveness or harm.

TOXIN:

A poison from natural sources (microbes, plant or animal) that usually is unstable, requires an incubation period, and is antigenic.

TOXIC:

Obviously, this word is the heart of toxicology. Toxic, in the medical sense, applies to the nature of a poison, and implies a lethal property. All things, in sufficient amounts, are of course toxic. In the popular sense the word toxic suggests the generation of undesirable side-effects.

ACUTE:

In the diagnostic sense, having a short and severe course. Acute appendicitis, for example. In drug use, having a single exposure. Acute cocaine exposure, for example.

CHRONIC:

Long continued, again and again. Subacute is a fascinating word that means something in between acute and chronic. With drug exposure, it usually means that things have been used for a short while.

POISONS FROM THINGS:

SENSITIVITY TO THINGS:

Antigen-antibody interaction. The exposure to a big thing, usually an alien protein or a polysaccharide, or a small alien thing that binds tightly to a natural big thing, producing an antibody that specifically recognizes and interacts with this thing. This concept will be discussed in Lecture #17.

Anaphylaxis. A sensitization seen on reexposure to this thing.

Ana- prefix meaning backwards or upwards, and
-phylaxis suffix meaning shield, Sensitization as opposed to immunity.

Pro- prefix meaning before – therefore a shield put up before exposure

Tachy- prefix meaning rapid – therefore a quickly established shield.

Allergy. A hypersensitivity to a thing that is harmless to most people. It may be obvious things such as pollen or dust, to less obvious things such things as cold or heat, to light, or even to a mental or emotional state.

POISONS FROM MICROORGANISMS:

Why do bacteria harm you? There is either a bulk-action – so many that they represent a space invasion problem, or there is the secretion of toxins (endotoxins if contained within the bacterium until rupture, or exotoxins if secreted by the bacteria while intact). These excreted toxins are usually proteins or polysaccharides of extreme potency.

In botulism, the toxin is a protein with an LD50 (in mice) at a dose of about a picogram (a millionth of a millionth of a gram). The organism is not needed, only the toxin.

In tetanus, the organism enters, then releases the toxin which specifically affects the spinal cord.

In diphtheria, the organism enters the respiratory tract, and the released toxin affects many organs.

In gas gangrene, the organism enters and releases a toxin that destroys tissue and allows the further invasion of bacteria.

Enterotoxins are toxins that arise within the intestine.

In cholera, the organism invades the gut, and liberates an endotoxin containing polysaccharides. Interestingly these are largely pathogenic only in man.

In food poisoning, usually there is an ingestion of Salmonellae or Shigellae which lodge in the gut and release toxins, largely lipopolysaccharides, which lead to dysentery, diarrhea, or tenesmus (a futile urge to empty rectum or bladder).

POISONS FROM ANIMAL SOURCES:

A venom is a poison secreted by an animal. Some animals secrete complex

alkaloids that are highly toxic; these are usually classified as poisons rather than venoms. The term venomous animal usually refers to an animal having a gland, a duct, a structure, for delivering its venom. The term poisonous animal usually refers to an animal which must be ingested to be harmful.

Histolytic, from histo- (tissue) and -lysis (to loosen or dissolve), is a venom that attacks muscular tissue.

Neurolytic, from neuro- (nerve), is a venom that attacks the nervous system.

Ophidism, from ophis (snake), having been bitten by a snake.

INSECTS AND SPIDERS: The toxic products of insects and spiders are transmitted to man

By stinging:	ants, bees, wasps, scorpions;
By bite:	mosquitoes, spiders:
By hair contact:	caterpillars;
By vesicating secretions:	blister beetles.

ANTS: Ant venom is very painful, and causes edema and cardiovascular effects. The chemical nature is not known.

BEETLES: The vesicant action (vesication, the process of blistering) of some beetles is well known, but it is usually due to simple compounds rather than to venoms. The Spanish fly produces cantharidine (irritating at 100 micrograms) and famous for its supposed aphrodisiac properties. Any apparent genital stimulation is the result of blistering irritation. The African beetles of the genus Zonabris produces both dermatitis and conjunctivitis (Nairobi eye). The coconut beetle in the mid- Pacific produces both pain and blister, and the Amazon is well supplied with vesicating species.

BEES: Bee venom is a protein which is relatively non-toxic in an unsensitized person. However, bumblebees produce a larger amount of toxin, and the giant bees (the bambarra of India, or the mangavas of Brazil) can kill large mammals. The best studied toxin is the protein, mellitin, with molecular weight of about 3000. It is neurolytic.

CATERPILLARS: The larvae of many butterfly species have urticating hairs (urticaria, a skin irritation like nettle rash, or hives) that can cause contact swelling and be quite painful. It is probably a protein. It is possible that the adult body of some moths and butterflies can cause contact dermatitis.

FLEAS AND MOSQUITOES: The toxicity here is probably due to antigenic properties. The various disease vectors are a separate discussion. The common bed-bug probably secreted an anti-coagulant enzyme. This is not a venom.

SPIDERS: The two most notorious spiders in our part of the world are the black widow *Lactrodectus mactans* and the brown recluse or the fiddle spider *Loxosceles reclusa*. The black widow bite is felt as a sharp pin-prick, followed by a painful neurological involvement lasting for a couple of days. The recluse bite is a histolytic problem leading to necrosis (tissue death) and ulceration (an ulcer is an open sore other than a wound). This spider is known here on the West Coast as far north as Santa Barbara, and has been recently reported in Florida.

The scorpion secretes a neurotoxic proteinacious venom that is toxic in mammals at the few micrograms/kilogram level.

The tick can release a salivary secretion which is neurologically complex and causes a paralysis taking many days to express itself. Removal of the tick quickly dispels the symptoms. A recent health problem is becoming quite severe in parts of California. This is Lyme's Disease, caused by the bite of an almost invisibly small tick. The signs of a bite are usually well characterized, and prompt treatment can be very valuable.

POISONING BY SNAKES:

Almost all snakes produce some sort of venom, but most of them can't bite you because their teeth are slanted the wrong way for skin puncture. There are four families, with names based on the location and the markings of these teeth. The word glyph means something carved.

Aglyphae – A- (meaning without) – snakes without teeth (originally Aglyphodonta, meaning no carved teeth). Since there are no teeth, there are no poisonous teeth, but many of them still have poison-secreting glands.

Opistoglyphae – Opisto- (from opisthen, meaning behind) – snakes which have poisonous teeth, but they are at the back of the jaw and cannot inject this poison upon biting.

Proteroglyphae – Protero- (meaning earlier) snakes having grooved teeth at the front of the jaw, and one that can secrete poison.

Solenoglyphae – Soleno- (from pipe, related to syringa, to inject) snakes with needle-sharp mobile teeth.

The last two families are toxicology problems. The proteroglyphae are the sea-snakes and the cobra snakes. The solenoglyphae include the vipers and the pit-vipers. They consist of over 400 species. In general there is no dependable sign of having been bitten by a snake. The sea-snakes give a painless bite, and there is only the slow development of paralysis with eventual respiratory failure. The cobras and vipers, however, give a burning, piercing pain at the bite-wound, followed shortly by edema. All snake venoms are relatively low molecular weight proteins in a complex mixture, but they also contain appreciable quantities of purines, metals, and enzymes. Most

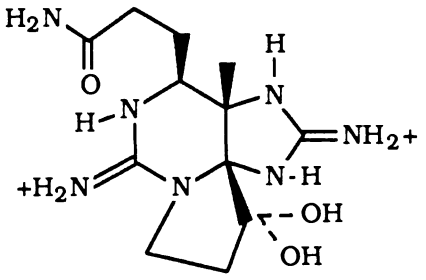
venoms exhibit a curare-like effect on muscle action.

POISONING BY MARINE ORGANISMS:

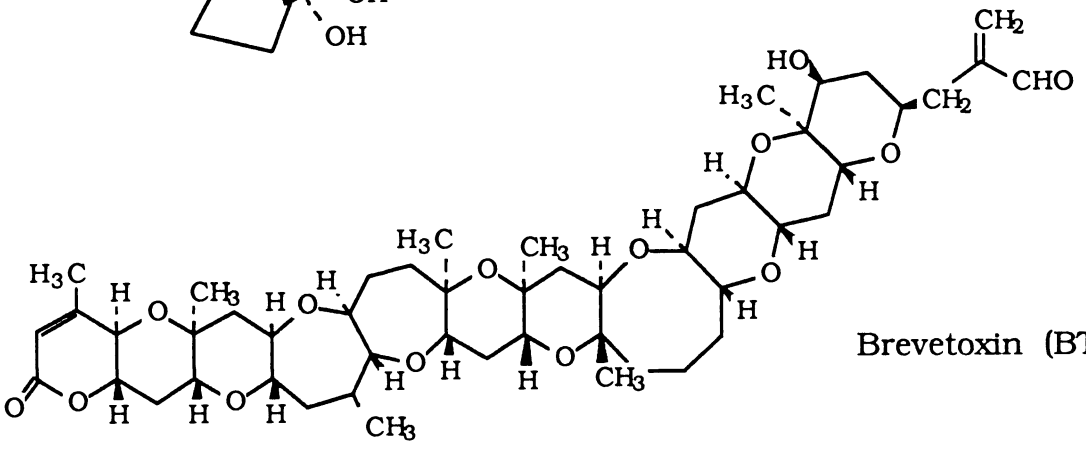
Most marine animal poisoning has been assigned to toxic alkaloid-like compounds rather than to venoms.

SHELLFISH POISONING:

Mussels and clams can be toxic due to ingested dinoflagellates. In the mussels, the toxin is largely in the digestive gland; with the clam, largely in the siphon. It is also found in the butterclam and the scallop. It is an actual metabolite of the dinoflagellate, and not of bacterial origin. It is the agent associated with the red tide seen along the California and Alaskan coasts, and a major active chemical factor is the water-soluble sodium channel blocker saxitoxin or STX (see structures). A similar red tide observed in the Gulf of Mexico and along Florida, is a different dinoflagellate that contains a lipid-soluble channel-blocker Brevetoxin (BTX)



Saxitoxin (STX)



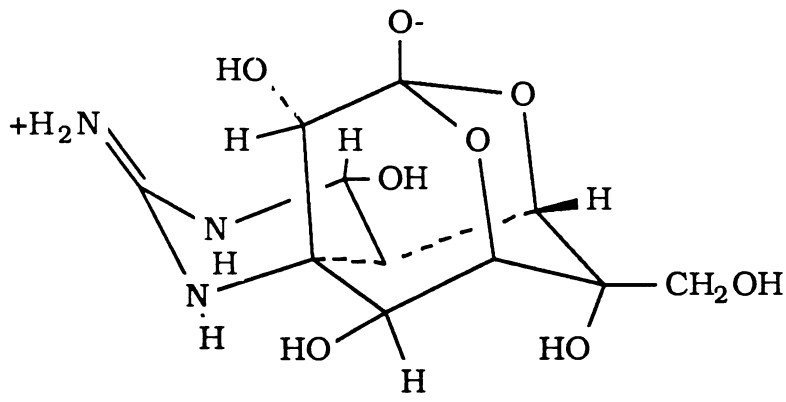
Brevetoxin (BTX)

Sponges, star-fish, sea-cucumbers, crayfish and anemone give painful poisoning, but the mechanics and chemistry are not well studied. The conus snail has a true venom, which is a protein or is bound to protein.

FISH POISONING:

About 500 species of fish are known to be toxic, or may on ingestion be poisonous to man.

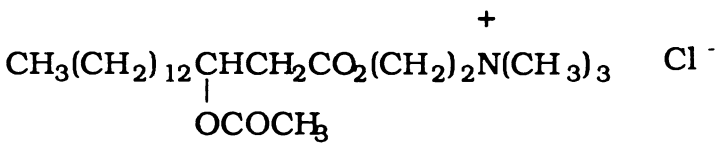
Tetrodotoxin (or tarichatoxin, or TTX) is found in the fugu poisons, and is contained in certain puffers, ocean sun-fishes, and porcupine-fishes. The poison



Tetrodotoxin (TTX)

produces a complete nerve-muscle blockade, and is responsible for about 100 deaths a year in Japan. There is a rapid onset of dizziness and pallor, often with a numbness all over and a feeling of "floating in air." Death occurs in 6 - 24 hrs following ingestion of the fish. Its LD-50 in mice is 10 micrograms/Kg i.p.

There are many other reported fish toxins: Ciguatoxin (or ciguatera poison) is found in normally edible fish, including barracudas, sea basses, snappers and moray eels, and is a quaternary amine affecting neuromuscular transmission; Saurine is a poison associated with a "peppery taste" reported when eating mackerel, bonita, or tuna. Poisonings have been regularly observed from lamprey flesh, and certain shark liver at certain times. Certain mullet and goatfish, upon ingestion, regularly lead to a hallucinogenic and intoxicated state called "nightmare week." The boxfish (trunkfish) releases the toxic principle Pahutoxin probably as a defense mechanism.



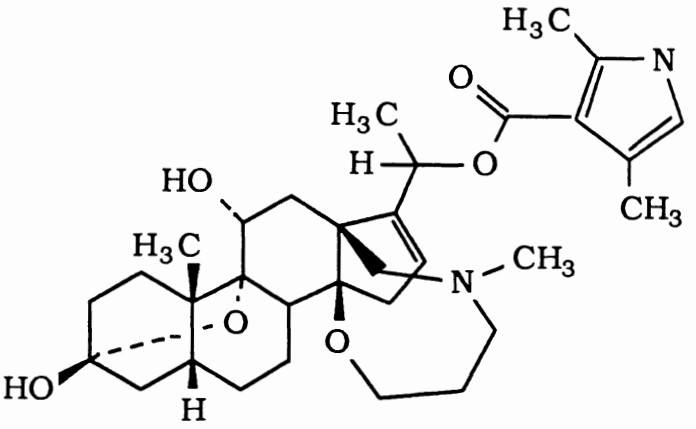
Pahutoxin

The stingray's venom is a protein with cardiovascular pharmacology; the scorpionfish and stonefish exude a proteinacious venom which is lethal to mice (precipitous arterial blood pressure drop) with an i.v. injection of about 5 micrograms.

FROG POISONS:

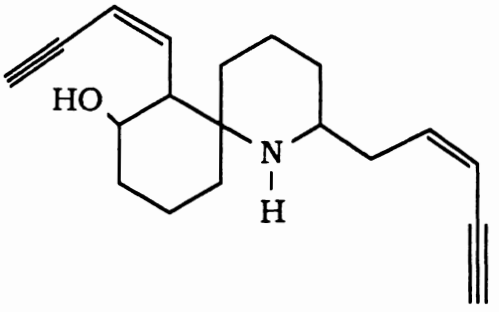
One of the richest sources of poisonous alkaloids from animal sources has been the frog. The most complex and toxic come from the Colombian arrow frogs of the

Genus *Phyllobates*. The structure of the steroidal base Batrachotoxin is shown, and its LD50 in mice is 2 micrograms/Kg, or about 50 nanograms per mouse, i.v. It is

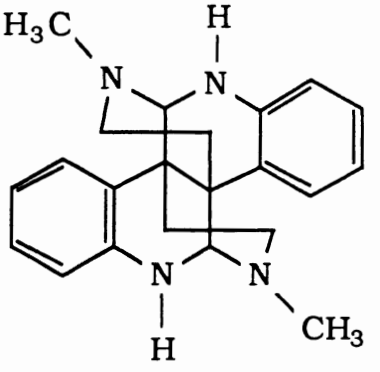


Batrachotoxin

interesting that the dimethyl-pyrrole carboxylic acid moiety is essential for biological activity. The alcohol counterpart (BTX-A) is 500x less toxic. Another species, *P. terribilis* gives rise to a pair of alkaloids identical to, but of opposite rotation to, the Carolina allspice toxic alkaloid, Calycanthine.



Calycanthine



Histrionicotoxin (HTX)

Another highly toxic alkaloid from a related poison-frog *Dentrobates histrionicus* is the toxin Histrionicotoxin, (HTX).

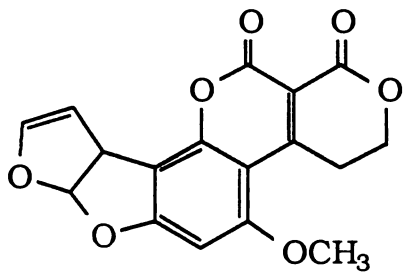
POISONING BY MUSHROOMS

The term "fungus" is quite generally used to include primitive plant life lacking usual plant morphology (roots, stems, leaves) and lacking chlorophyll. It includes molds, rusts, mildews, yeasts, and mushrooms. The poisons from this group are called mycotoxins. It is interesting that most terms involving something-toxic refers to the thing being toxied against. Phytotoxins kill plants. Zoo-toxins kill animals. But myco-toxins don't kill fungus, they come from fungus. Some are truly toxins from the near-microscopic fungi, but most are alkaloids or peptides from their conspicuous and highly attractive fruiting bodies, the mushrooms. Poisonous mushrooms have

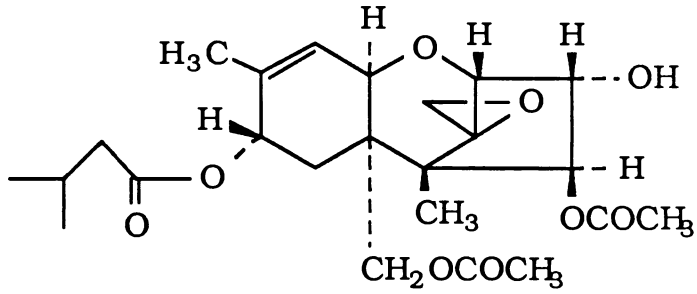
always been a topic of myth and social concern, and the term "toadstool" is popularly used to denote inedible mushrooms. The structures of the poisonous compounds isolated from mushrooms represent one of the most diverse collections imaginable. Interestingly, most toxins from molds do not contain nitrogen, whereas most poisons from mushrooms do. A few of these toxins and poisons are illustrated.

The aflotoxins represent a closely related group of highly carcinogenic metabolites of the mold *Aspergillus flavus* known best for its occurrence on peanuts. They are named with letters describing their fluorescence (B for blue, G for green) or their sources (M for milk) and numbers for their relative mobility by TLC. They are also quite toxic (1 or 2 mg/Kg). They are inhibitors of nucleic acid synthesis.

The Tricothecene mycotoxins are also non-nitrogenous, and have received



Aflotoxin G_1

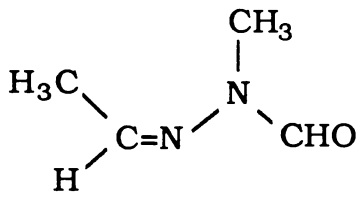


Mycotoxin T-2

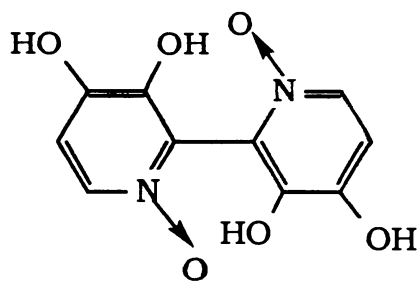
broad notoriety as chemical warfare agents (see lecture #4 for the various military codes and trivial names). They are produced by the Fusarium molds. Contact produces rapid bleeding (and bloody diarrhea), vomiting and dizziness. The "yellow rain" ascribed to the Soviets in Southeast Asia has been identified by some as containing Mycotoxin T-2, but has been identified by others as being nothing but bee feces.

At the poison mushroom level, three examples show the broad structural diversification possible.

The false morel *Gyromitra esculenta* is not easily mistaken for the true morel *Morchella esculenta*. The latter is edible, but the former contains the poison Gyromitrin, a deceptively simple formyl hydrazide. Remember, if the top of it is pitted,



Gyromitrin

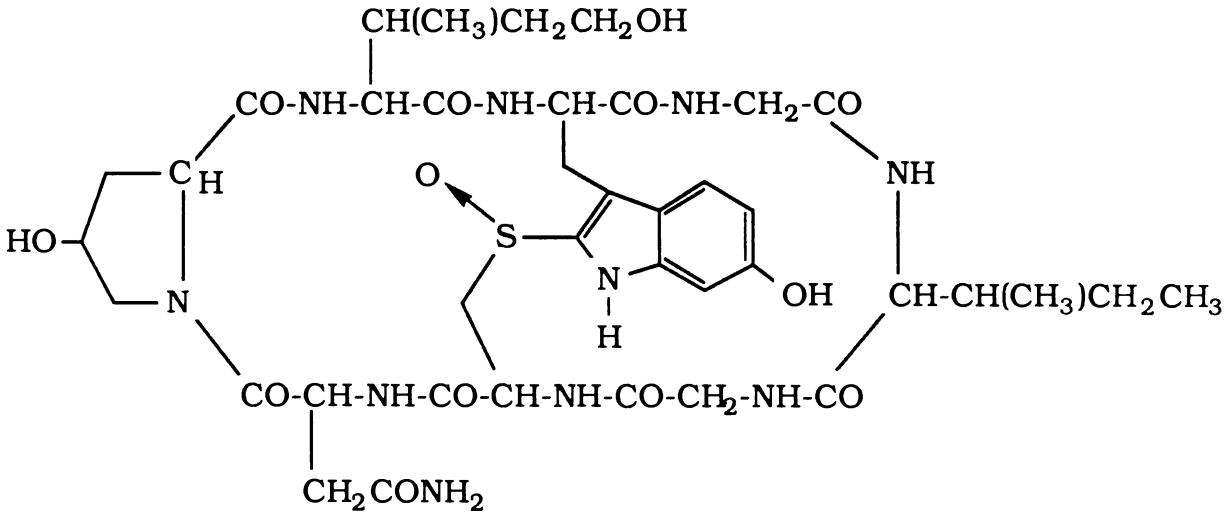


Orellanin

like a honeycomb, it's OK. If the top of it is convoluted like brains, not OK. If in doubt, don't.

An interesting recent discovery are the toxic species of the Genus *Cortinanus* which grow native to Poland. The poison is Orellanine, a bipyridyl N-oxide with a three-dimensional structure that cries out to chelate some vital metal. This mushroom emphasizes a common difficulty in identifying plant with poison; there is a lag period of up to 14 days noted between the eating of the plant and the onset of symptoms.

And then there are the fascinating cyclic polypeptides from the famous mushroom *Amanita phalloides* or the "destroying angel." Two groups are well established, the Amatoxins (the more toxic but slower in action, and all containing a sulfoxide group despite the erroneous entries under amanitin in the Merck Index) and the Phallotoxins (the less toxic, faster acting, and without the sulfoxide group,



gamma-Amanitin

correctly drawn under phalloidin in the Merck Index). gamma-Amanitin, shown here, will kill a mouse with an injection of about 5 micrograms, and in human poisoning, even if there is survival, there is often long-lasting liver and kidney inadequacy.

POISONS FROM PLANTS

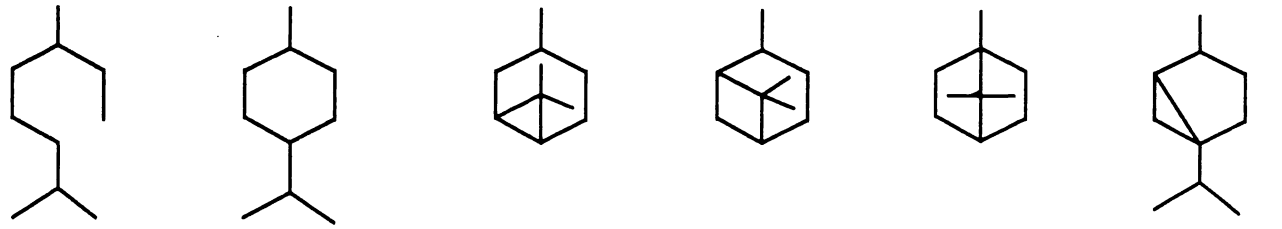
The science of chemotherapy (the use of drugs to treat illness, and the science of pharmacology (the action of drugs on living organisms) have their origins almost entirely in the area of botany. Almost every drug known can be traced back to some natural compound found in a natural plant. It is outside the scope of this review to give examples of everything. A few general classes will be portrayed, with representative examples.

TERPENES.

A basic building block in plant chemistry is isoprene, the five-carbon diolefin

2-methyl-1,3-butadiene. Natural plant polymers such as rubber yield isoprene on destructive distillation, and isoprene on being shaken with conc. HCl repolymerizes to a rubber-like mass. The Germans in the 1920s explored both butadiene itself, and 2,3-dimethylbutadiene, as starting materials for synthetic rubber.

Two isoprene units, hooked head to tail, constitute the 10- carbon unit $C_{10}H_{16}$ which is called a mono-terpene (or sometimes simply a terpene). This dimer occurs as the open chain form dipentene, the single ring form menthene, or in any of four bicyclic forms carene, pinene, camphene and sabinene. These basic terpene families



Dipentene Menthene Carene Pinene Camphene Sabinene

are illustrated. If three isoprenes are linked, the C_{15} unit is called a sesqui-terpene, and on as follows:

C-15	Sesquiterpene	(sesqui-, 1-1/2 times)
C-20	Diterpene	(di- two of them)
C-25	Sesterterpene	(sester-, 2-1/2 times)
C-30	Triterpene	(tri-, three of them)

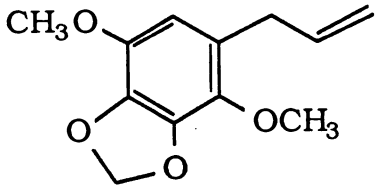
Many of the plant polymers are polyterpenes, and this origin can be seen in the so-called isoprene rule, in which a complex molecule can be somehow gone about, carbon by carbon, with a one, two-bump, three, four, where the "bump" is the branched methyl group (at least in its origins). (Look up the structure of the carotenes and try this out! Where and why does it go wrong?)

ESSENTIAL OILS:

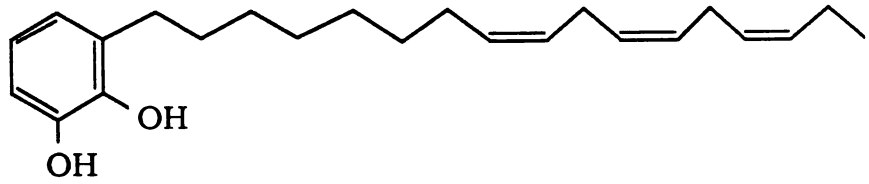
This large class of plant chemicals have the name essential pertaining to smell rather than to importance. These are usually phenylpropenes, and serve as the major components in spices and flavors. As spices and food-flavorings they have been invoked in lay-medicine for generations, and it is not surprising that there is a large body of literature about their uses and misuses. Apiole (a major component from parsley) is illustrated. With most of these oils (for example safrole, elemicin, myristicin, asarone, etc.) the addition of a molecule of ammonia to the double bond (a reaction that has been shown to occur in perfused liver) produces a centrally active amphetamine analog.

NON-NITROGENOUS PHENOLS

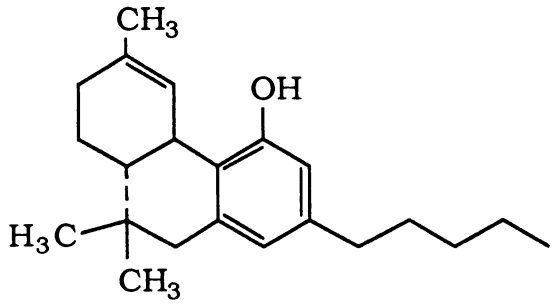
A number of 3-alkyl-catechols, with various degrees of unsaturation are known components of poison oak (17-carbon) and poison ivy (15-carbon). They are collectively known as the Urushiols. The poison sumac has similar chemistry. 5-Alkylresorcinols are widely spread in nature. Some with a long odd-carbon chain demand attention as irritants (as with cardol, from the cashew-nut shell oil, with a 15-carbon



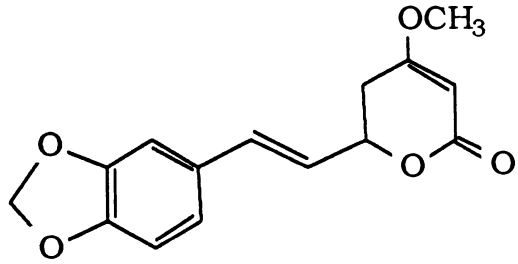
Apiole



Urushiol



delta-1 THC



Methysticum

chain). The five-carbon chain (and to a lesser degree, the 3 and the 7- carbon chains) have been immortalized as the backbone of the THC molecule. This is a CNS-active intoxicant, and a major active contributor to the action of the marijuana plant.

Methysticum is another non-basic intoxicant, a major component of the "narcotic pepper" *Piper methysticum* or the Kava- Kava of the South Pacific. It and Kawain are the probable active ingredients, and these are currently available in Germany as sedatives.

POLYPEPTIDES AND PROTEINS:

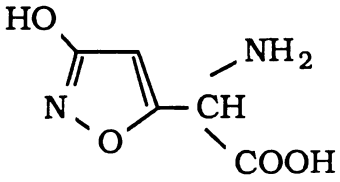
There is an unending parade of peptides and proteins that are from natural sources and that are toxicologically important. The shorter peptides are known as sweetening agents, as narcotic analgesics, as releasing hormones, and as biologically active units which can catalyze extraordinary bodily changes. The intermediate peptides have been mentioned as Amanita toxins. Fully toxic proteins are many, such as ficin (the protolytic enzyme from the latex of the fig), papain (the active enzyme from the papaya latex), bromelain (the digestive enzyme from the pineapple) and ricin (a

hemagglutinin isolated from the castor bean). This latter protein is lethal in mice at about 2 nanograms i.p.

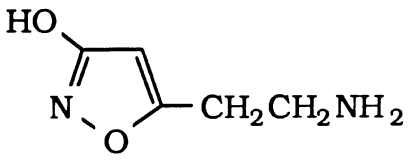
ALKALOIDS:

The alkaloid family is without doubt the largest single class of chemicals, from plant sources, known to man. An alkaloid is usually defined as a nitrogenous organic base that occurs in plants. It must contain nitrogen and be a base. It must be somewhat complex. But the association with plants rather than animals is a fuzzy distinction. There are literally thousands of alkaloids, of hundreds of chemical families, and with innumerable examples of biological activity (toxicity). Again, examples will be given of only certain chemical classes, to give the flavor and music of the many diverse classes of structures that are known to have pharmacological activity.

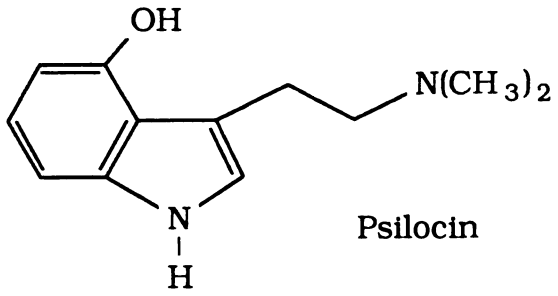
There are two popularly acknowledged "psychedelic" mushrooms. *Amanita muscaria*, the fly agaric, contains two GABA- related compounds, ibotenic acid and



Ibotenic Acid



Muscimol

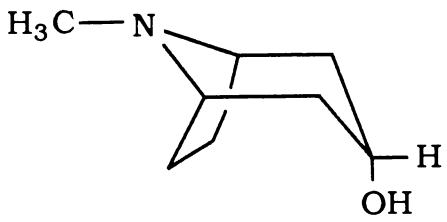


Psilocin

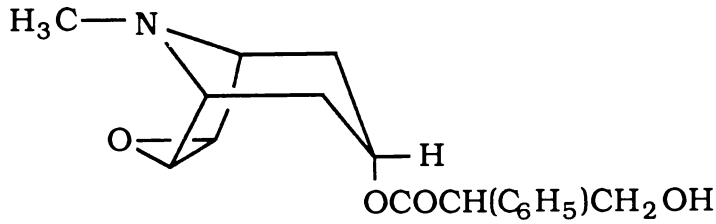
muscimol. These toxins have been assigned responsibility for the activity of the parent fungus. And in the many species of the Genus *Psilocybe* or *Panaeolus* one finds the hallucinogenic compounds psilocin and its phosphate ester, psilocybin.

The tropane group contains a bicyclic piperidine common to a number of fascinating compounds. The parent ring system is tropine and pseudotropine, both alcohols despite the -ene name. A triple-A mnemonic is useful here, to keep orientations straight. The structure that is atropine is called alpha and is axial. A,A, and A. Atropine and scopolamine are direct consequences of that orientation at position 4. On the other hand the other isomer is that of cocaine, it is the beta orientation, and it is the equatorial isomer of this structure. C,B and E, rather than A,A and A. Note that in scopolamine (and in atropine) the hydroxyester to the tropic

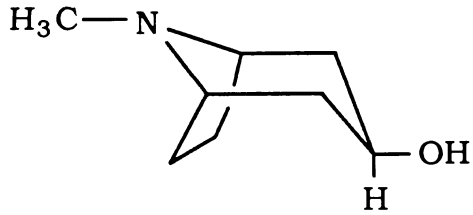
acid moiety is down, and in cocaine, the hydroxyester to the benzoyl moiety is up. Therein lie the pharmacological differences.



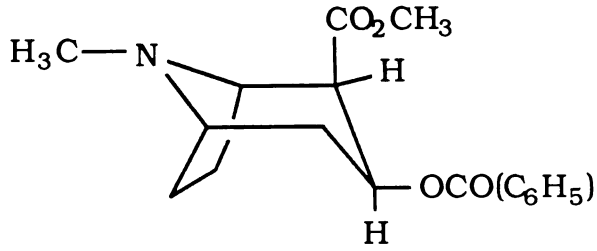
Tropine



Scopolamine

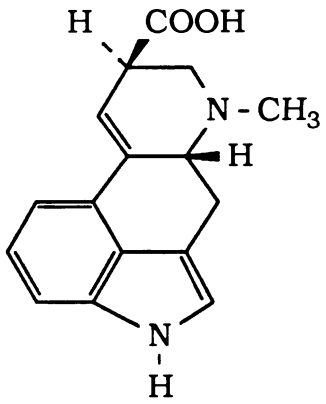


Pseudotropine

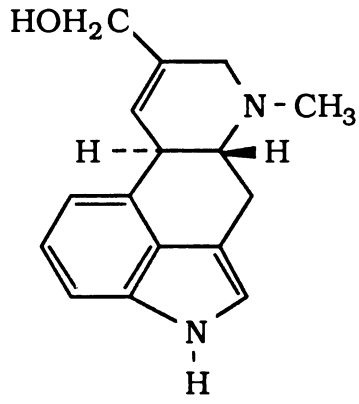


Cocaine

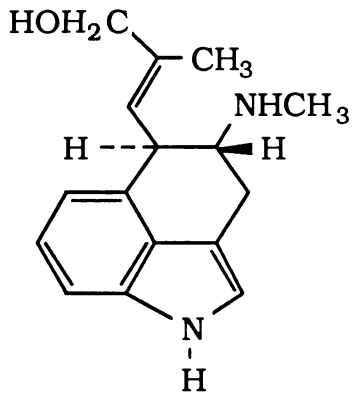
With the alkaloids of the *Ipomoea* (these are the ergot-like compounds related to the lysergic acid group, and thus to LSD) there are three families, in which the "D"



Lysergic Acid



Elymoclavine



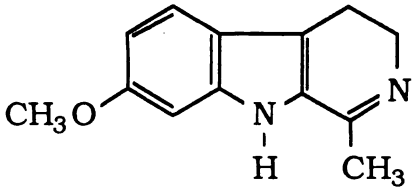
Chanoclavine

ring of LSD is structurally distorted. All of the lysergic acid compounds have an intact D-ring, but the related alkaloids can have this ring open, and the double bond is relocated. The two chiral centers of lysergic acid can give rise to four isomers.

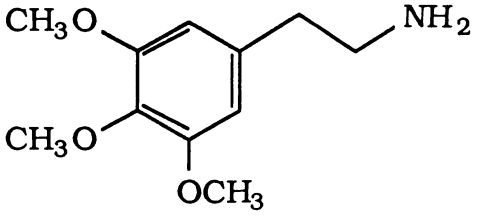
The *Banisteriopsis* group of South American intoxicants contain harmaline as a major ingredient. Here is an interesting example of jungle psychopharmacology. Harmaline is only a modestly effective intoxicant, but it is a potent inhibitor of the enzymes that oxidize amines (monoamineoxidase inhibitor, MAOI). Also in the northern parts of South America, there is a snuff used, called Epena or Parica (largely

from the *Virola* genus), that contains dimethyltryptamine (DMT) as one of its major active ingredient. But this plant material is classically used as a snuff for the straight forward reason that it is not active orally. And yet some tribes have combined these two plants allowing DMT to be effective orally. Indoles related to DMT have been explored in the central nervous system (CNS) because of their close structural relationship to the neurotransmitter serotonin.

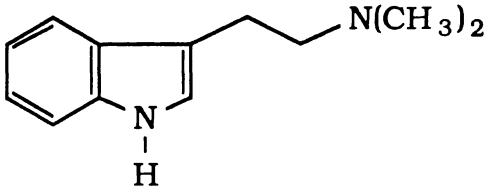
The dumpling cactus *Lophophera williamsii* or Peyote, contains many dozens of alkaloids, the principal one being mescaline. This deceptively simple molecule is a



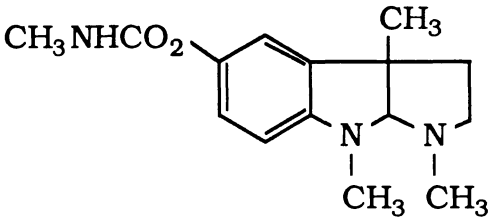
Harmaline



Mescaline



Dimethyltryptamine (DMT)



Physostigmine

complex hallucinogen, and has served as a starting point for the development of research probes that are structurally most closely related to the neurotransmitter dopamine.

To round out the neurotransmitter picture, the structure of physostigmine is given. This is a potent cholinergic drug in that it inhibits the acetylcholine esterase system. Again, an interesting bit of native psychopharmacology. In certain tribes in Africa, there is practiced what is called "trial by ordeal." When a person has been accused of a capital crime, he is given several seeds of the plant *Physostigma venenosum* (Calabar beans) to determine his guilt or innocence. This seed contains several potent alkaloids, one as mentioned above, the murderous cholinergic physostigmine, but another is an effective emetic (causing vomiting). If the defendant is innocent, he clearly knows he will survive and swallows the Calabar beans. The emetic is absorbed and all is vomited out. If he is guilty, however, he will show nervousness and hesitancy about eating the seeds that will show that guilt, and the physostigmine is absorbed from the mouth while he is chewing and worrying, and he is effectively executed.

MAN-MADE POISONS:

INDUSTRIAL POISONS

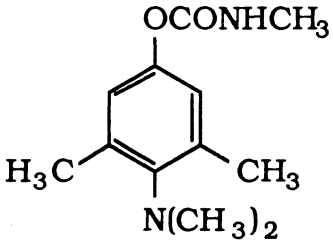
Insecticides are a common source of poisoning. Most insecticides are choline esterase inhibitors, similar to the activity of physostigmine in the last lecture.

Acetylcholine (AcCh) is a neurotransmitter. Most nerves do not touch one-another but are separated by a narrow gap called the synaptic cleft. Thus for one nerve to inform its neighbor that there is a message, it must release a little bit of a chemical that will drift across this gap. This chemical is called a neurotransmitter, and its identity gives name to the system of which the nerves are a part. Thus one speaks of the cholinergic nervous system (more properly, the acetylcholinergic nervous system) when acetylcholine is the needed neurotransmitter.

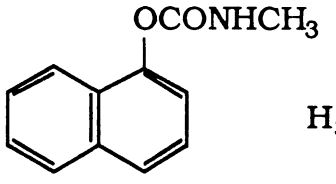
But once the communication has been achieved, the acetylcholine must be removed, otherwise the receiver nerve will be continually excited leading to sustained muscular contraction (if the nerve controls a muscle). The way the body does this is to hydrolyze acetylcholine to choline, which is ineffective as a neurotransmitter. This saponification is achieved by an enzyme called acetylcholine esterase (AcChE). Obviously the more enzyme there is present, the less effective is the neurotransmission, and the less enzyme, the more effective.

Most insecticides, and for that matter most nerve gases, which will be pointed out later in the lecture, are inhibitors of this enzyme; i.e., acetylcholine esterase inhibitors (AcChEI).

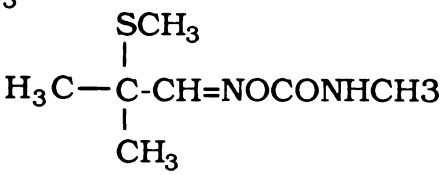
There are two ways of inhibiting this enzyme. A compound can fit into the active site effectively for a while, but not do anything. But while it is there, no acetylcholine can get in. Hence the enzyme is ineffective, but in time the inhibitor does indeed get saponified, and the enzyme is once again available for its correct job. This is called reversible inhibition. Most of the commercial insecticides that act in this way are carbamate esters. Illustrated are Zectran (which can be seen as an analog of physostigmine), Sevin, and Aldicarb. It is this last carbamate that inspired such a



Zectran



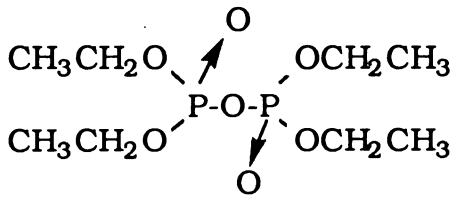
Sevin



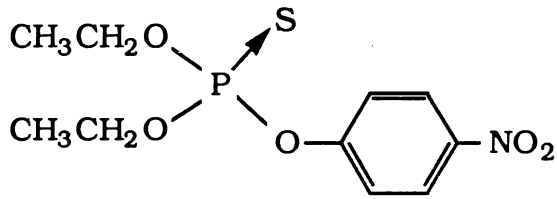
Aldicarb

recent public furor, both for its appearance in watermelons from the southern part of California, and with a release on the east coast (Union Carbide) of the uncarbamylated oxime. A chemical intermediate to all of these carbamate insecticides is methyl isocyanate (CH_3NCO). This liquid is an intense lacrimator, and was the agent involved in the Bhopal tragedy in India.

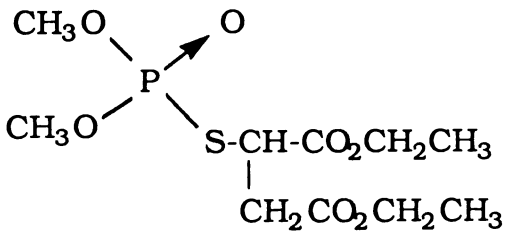
A second form of enzyme inhibition is irreversible. Here, there is a close association between the inhibitor and the enzyme, except that there is a chemical reaction, a true covalent association, and the enzyme has been permanently removed from further availability. Most of the phosphate insecticides and nerve gasses fall in



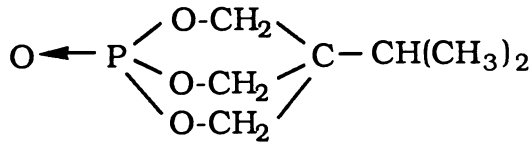
TEPP



Parathion



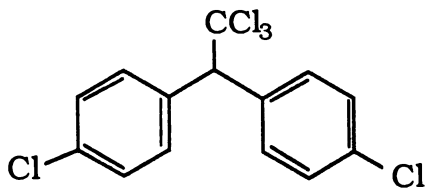
Malathion



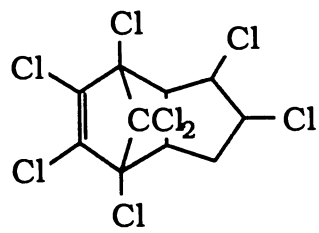
LD_{100} 200 µg/Kg

this classification. Three popular commercial insecticides are illustrated, as well as an unusual bridged phosphate of unusually high toxicity. This latter ester has a LD100 (in mice, I believe) of 200 micrograms/Kg. This makes a lethal dose less than 10 micrograms in an animal.

The chlorinated hydrocarbons represent another principal class of insecticides. Some of these are discrete compounds such as DDT, Chlordane and Dieldrin (illustrated). There are other products that have been commercially distributed and

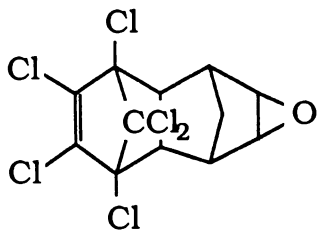


DDT

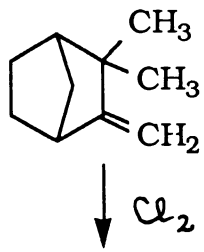


Chlordane

widely used, that are hopeless mixtures of largely uncharacterized compounds. For example, toxaphen is the chlorination product of camphene. It is a waxy yellow solid resulting from chlorination until there has been the uptake of between 67 and 69% chlorine by weight. There are over 175 compounds visible by high-resolution GC.



Dieldrin



Camphene

Toxaphene about $C_{10}H_{10}Cl_8$

Another, little explored toxicological problem with some of the chlorinated hydrocarbons that employ hexachlorobutadiene as a synthetic component in a Diels Alder reaction, is that this compound can under certain conditions give rise to the aromatic anion pentachlorocyclopentadienyl which has been reported to have a high toxicity. A further problem is that many of these highly chlorinated hydrocarbons can be absorbed quite readily through the skin.

Polychlorinated biphenyls: There has been much publicity given recently to the environmental distribution of the PCBs. In the 1930's it was found that the products obtained from the chlorination of biphenyl could be exploited commercially. They were good insulators, had very low vapor pressures, were good fire retardants, and especially, were unusually stable. These very properties are now appreciated in their pervasiveness in our environment. They are now known to be carcinogenic, to reduce ability to reproduce, to cause mouth and eyelid edema, to decrease hemoglobin, and to cause chloracne (a generalized skin eruption brought about by contact with highly chlorinated compounds). The accidental contamination of rice oil with Kanneclor 400 (a PCB mixture) in Japan led to the outbreak of what is now known as "Yusho disease." The compounds in the mixtures are not terribly toxic (typical toxicity more than a gram/Kg orally in rats) but they are subtle in their long term effects, and they are virtually indestructible.

The analogous family with bromine rather than chlorine (polybrominated biphenyls, or PBB,s) have been commercially available as fire-retardants. Firemaster BP-6, largely a hexabromobiphenyl, was the culprit in the 1973 "Michigan Incident" where it was interchanged with a food supplement, and accidentally added to cattle feed. The consequential mandatory wide-spread destruction of contaminated farm animals led to the removal of BP-6 from the market.

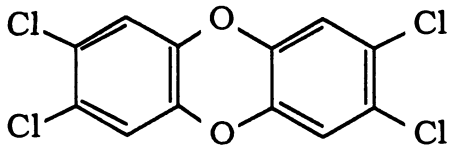
Chlorinated dioxins: This is another class of dangerous substances. The principal target for study is 2,3,7,8-tetrachlorodibenzo-para-dioxin, or TCDD. Its first environmental introduction was as a by-product in the manufacture of 2,4,5-trichlorophenol, itself a precursor to the herbicide 2,4,5-T. It has been implicated as the causative agent in the long-term health problems experienced by veterans exposed

to Agent Orange. The toxicity of TCDD is extremely variable, depending on the animal employed, with the LD50s ranging from (orally) 1 microgram/Kg in the guinea pig, to 5 mg/Kg in hamsters. The human toxicity can only be gleaned from accidents. The most notorious was the 1976 Sevesco (Italy) release where a cloud of about 2 Kg drifted over and settled onto a small neighborhood. Although thousands of herbivorous animals died, no humans did, so the acute toxicity to man must be quite low. This is another very stable and persistent agent in our environment. A black sideline to this event was the packaging and disposal of 41 barrels of the most highly contaminated material. It left Italy shortly after the dispersal, and appeared, unannounced, behind an unused slaughter house in Paris the following May. No one claimed responsibility.

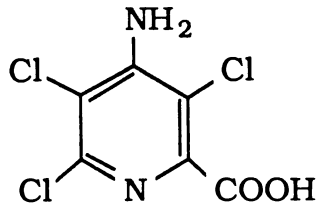
CHEMICAL-BIOLOGICAL WARFARE AGENTS:

Much of the government's research efforts have been directed to the development and refinement of the so-called CBW agents. Although most of this information is not public since it is usually put into some form of security classification, a careful gleaning of comments made in congress, and contract bid requests from the military, can allow a pretty complete picture to be inferred. In the illustrations given, names, structures and military codes are brought together in one place.

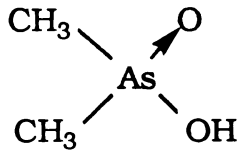
Herbicides:



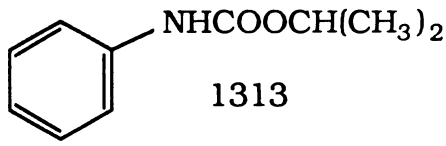
TCDD



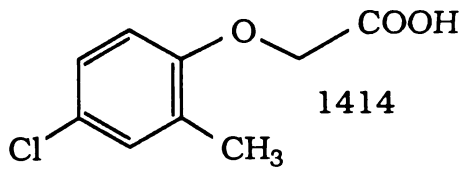
Picloram



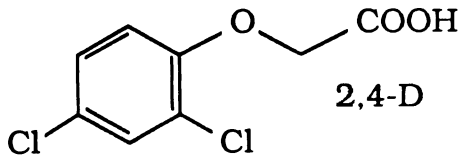
Cacodylic Acid



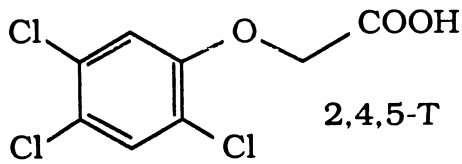
1313



1414



2,4-D



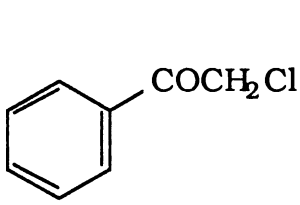
2,4,5-T

AGENT ORANGE	2,4-D + 2,4,5-T
AGENT WHITE	2,4-D + Picloram
AGENT BLUE	Cacodylic Acid

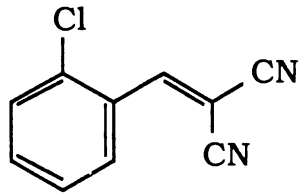
Mention has already been made of 2,4,5-T as being one of the original vehicles for the distribution of TCDD. The first development of herbicides as war weapons occurred in World War II with the discovery of two simple aromatic compounds, called 1313 and 1414. They were considered as potential destructive agents for the Japanese food crops. Three separate herbicides were widely used in Vietnam, under the names of Agents Orange, White, and Blue. The actual composition of these is listed above.

Harassing Agents:

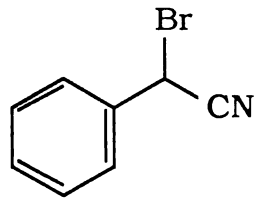
The concept of a harassing agent is to provide a transient, reversible incapacitation, either through physical disturbance (sneezing, vomiting, lacrimation) or mental disturbance (confusion, hallucination, sensory distortion). It is a fascinating story to follow how our culture has benefited (?) from CBW research. The three principle physical harassing agents are shown. "CN", phenacyl chloride, a very effective and very fast acting eye, throat and skin irritant, is now a mainstay in police arsenals as a riot-control agent. And it has come into the home as a purse-carried defense under the name of Mace and Curb. "CS", however, is less fast, less incapacitating, but considerably more irritating, than "CN". It is now in many police arsenals for potential riots, and in many ladies' purses with the brand name Chem-



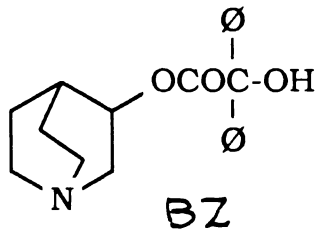
CN



CS

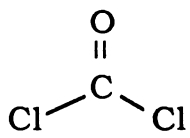


CA



BZ

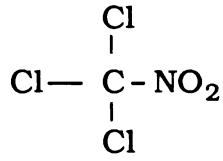
shield. Bromobenzyl cyanide "CA" is apparently still of interest only to the military. It is primarily a lacrimator, and has been studied in combination with such things as chloropicrin (CCl_3NO_2, a respiratory irritant) and phosgene. At the physical distur-



CG (Phosgene)

HCN

AC (Prussic Acid)

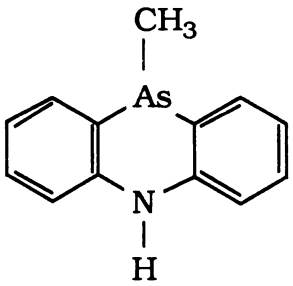


PS (Chloropicrin

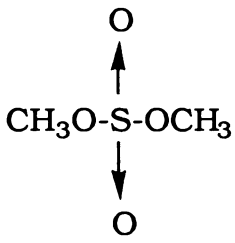
bance level, the record is much more interesting. Many of the materials that were been developed and studied by the Army (the main research site was and still is the Edgewood Arsenal, in Maryland; the biological research center is known as Fort Detrick) were things that are now commonplace "dangerous" drugs. The early studies with LSD grew into the MKULTRA scandals of the CIA in and about 1952. Much of the hospital-conducted clinical work with LSD was quietly funded by the CIA. It was not until the early 1960's that the role of LSD in social upheaval became apparent. The first human studies on phencyclidine (PCP) were conducted with Army volunteers before 1960; PCP appeared as a social problem in June, 1967, when it was sold as THC on the streets of San Francisco. And speaking of THC, the military funded an immense research project at the Arthur D. Little Company in Boston, to produce kilogram quantities of it. "BZ" was a me-too project, since it was known that the Russians were deeply invested in researching it. It is felt by many writers that the military and especially the CIA set loose the forces that led to the drug culture of the 1960's.

Incapacitating Agents:

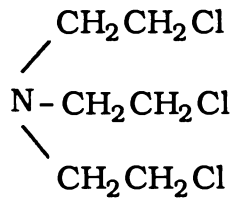
These are agents that have a sufficiently long action that hospitalization and



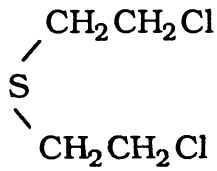
DM (Adamsite)



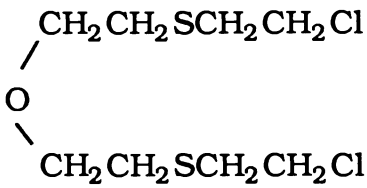
Methyl Sulfate



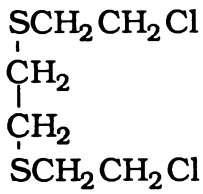
HN3 (60 μg)



HD (32 μg)



T (4 μg)

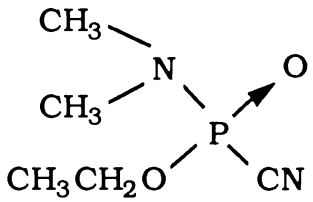


Q (0.3 μg)

extensive medical care would be required. Both Adamsite "DM" and methyl sulfate were World War I factors, but the development of some of the illustrated vesicants represents more recent research. Four of these mustards, demonstrating the wide range of potencies, are illustrated. The weight given is the amount which when applied to human skin, will cause blistering. Note that the potencies range over two orders of magnitude.

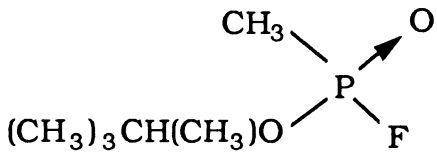
Lethal Agents:

The simple, crude, ponderous agents of WW-I (phosgene and HCN) have completely given way to the advent of nerve gasses, the volatile phosphate anticholinergics that had been developed in Germany in the 1930's and developed as weapons towards the end of WW-II. The Russians and the Americans swept through and grabbed everything visible in Germany at the close of WW-II, but the two major treasures were chemical information involving nerve gases, and technical information involving rocketry. So, for a decade following the war, the major powers held under tightest security wraps the structures of very simple phosphate esters which were extremely potent nerve gasses. Finally, a scientist in Scandinavia said "Enough. There are chemists around the world working in these areas who might be totally unaware of the extreme potency of these compounds. Here are their structures." And he published the lot – all of the secret information of Russia and of the U.S., and I don't know who else. Here are some of the structures of the principle weapons in both arsenals.



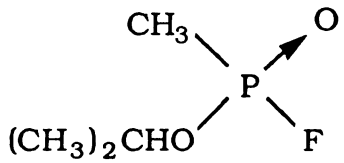
GA
Tabun

This nerve gas was developed in about 1936 by the Germans. It has the fruity smell of bitter almonds, and is water-miscible, but quickly hydrolysed.



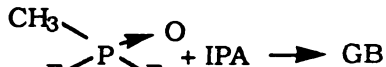
GD
Soman

Never successfully manufactured by the Germans, this is the current main stay of the Russian arcenal. It rapidly penetrates the blood-brain barrier. A thickened version, in Russia, is called VR-55.

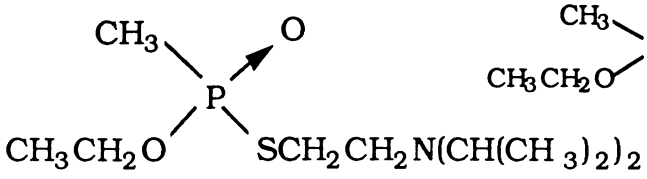


GB
Sarin

This is a binary gas made by the inter-

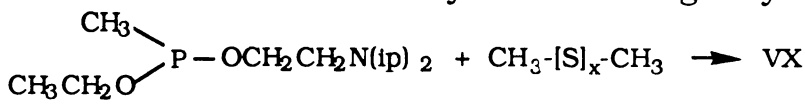


action, inflight, between a phosphorus acid fluoride (called DF) and isopropyl alcohol (code-named IP). The human LD-50 is 10 μg/Kg, and the current U.S. stockpile is about 30,000,000 lbs.



VX

This binary is made in flight by



the interaction between the ester-amine QL and a poly-sulfide code named NM. The human lethal dose is about 400 μg, and the present U.S. preformed stockpile is about 10,000,000 lb.

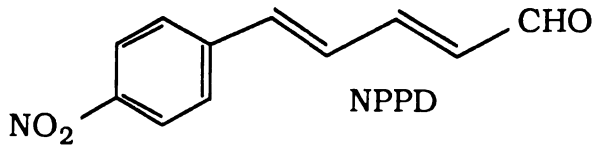
The binary concept is currently favored, in that, although there is some hesitancy in funding nerve gas research, there is none at all concerning the research into the biologically innocuous precursor components of nerve gases. These can mix in flight to form a valid nerve gas which, by present law, lies outside of political restrictions. And a little advertized but well documented chapter of World War II politics involved itself with the production (in Indiana), shipment (to England) and experimental deployment (fortunately not in anger) of bombs containing Anthrax

ANTHRAX SPORES	N	World War II production of 4 lb. bombs that were contained 100 to the projec-ile, manufactured in Vigo, Indiana, and shipped by the thousands to Britain.

spores. There are islands off the west coast of England that even today connot be inhabited, due to contamination from military bombing practice with test animals.

A little known complication involved in the nerve gas situation is that at the present time we have an inventory of out-dated munitions, including primaries, binaries, and components of binaries, that total approximately 100,000 tons. This must all be gotten rid of. Assignment: Bring to class next week an illustration of the mass, an image, a metaphor, a tangible gestalt, of 100,000 tons. And let us assume, as it is relatively old and maybe a bit inactive, it might take as much as a milligram to kill a man. Then, at 2 pounds to the Kilo, we are talking about 100,000,000 Kg. Or 100,000,000,000,000 mg or 100,000,000,000,000 human doses. And with maybe 5 billion souls alive on earth at the moment, that is 20,000 doses per living person. And if we can find a way (as promised by one contractor) to be 99.997 % effective in burning it, that would limit the acknowledged escape quantity to a mere 3 billion human doses. Downwind, of course.

And to end this part of the lecture on an up-beat, there was quite a flap a couple of years ago about the Russians using a spy-dust to track the movements of U.S. personnel in Russia. It is called NPPD or nitrophenylpentadieneal. Although there are only eight references to it in the Chem. Abstracts (seven Russian, one Australian), it is instructive to note that we had instant experts who are willing to say:



Nicholas J. Turro, a photo-chemist at Columbia:

"Its molecular structure is so simple you could have undergraduates prepare it in a high school lab. It is very likely fluorescent, and almost certainly a solid at room temperature."

Robert Michaels, a scientist with the Natural Resources Defense Council in New York;

"The carbon atoms probably make the chemical stick to fat molecules in the skin."

A State Department official, unnamed, said:

"The mutagenicity of NPPD has been determined using the Ames bacterial test."

Peter Andrews of the ACS in Washington said:

"It's reasonable to guess that the material may be mutagenic, but there's no reference to its mutagenicity in the published literature."

And the two final words come from congress:

Senator Strom Thurmond:

"I would go so far as to say that if they are going to put chemicals on our people over there, subject them to cancer, that we close the embassy entirely, if necessary. It's inhuman; it's barbarous; it's unreal."

Senator David Durenberger:

" — urge the expulsion from the United States all Soviet citizens affiliated with intelligence."

Is this chemical warfare? I certainly don't know.

RADIOACTIVITY:

Certainly, radioactivity is not a man-made poison, but the quantities that we are expected to encounter clearly come from the hand of man. And, remember, that all radiation is harmless until it interacts with something. It is this interaction that commands our attention.

What is the radiation that is encountered?

Alpha rays:

These are big, doubly charged helium nuclei that are slow, don't go far, but they do much damage. They are so big, so charged, so lumbering, that they dump all their energy on the first physical thing encountered, and if this happens to be a living cell, it is blown apart. Short range, but total destruction. But then they are mainly from nuclear bombs and plutonium and heavy things of that sort, and are not too much of a problem in the environment, unless plutonium is released into the environment.

Beta rays:

This is an electron that is moving very fast, but not quite up to the speed of light. Depending just where it comes from, it will have different speeds (different energies) and will do damage according to this speed (energy). And these beta rays (electrons) can impact matter in either of two ways. With low atomic number elements, the beta hits the orbital electrons, ionizing things, and probably produces H_2O_2 which is pretty rough on cells. With higher atomic numbers (or higher energy betas) they may make it to the nucleus of the atom, and can end up producing a bunch of X-rays (gammas).

Gamma rays:

This is a unit of massless energy, that zips along at the speed of light, until it is absorbed somewhere. Gamma rays (honest-to-God photons) can do any of three things. If the energy is very low: it can hit a nucleus and knock out an inner orbital electron, and when this is filled again, you get some X-rays but, of course, of lower energy. This is called the photo-electric effect. If the gamma hits a lower atomic number nucleus (the Compton effect) an outer shell electron may be knocked away, and as this is filled you might get yet more gamma, some of which might be photo-electric X-rays as discussed above. But very high energy gammas (above 1.02 mev) may hit a nucleus and eject an electron and a positron (called pair production) with an addition gamma or so, to balance the books. This is a direct conversion of energy to mass. The electron will be a kosher photoelectric electron (see above) but the positron will annihilate very quickly producing two gammas with annihilation energies, 0.511 mev.

To what extent are you being cooked? The unit of exposure is the Rad (100 ergs/g. water) but, with biological tissues, there is now the virtual equivalent, the Rem.

Let's rank Rems according to exposure, risk, and environment:

400	Rem at one exposure	probably lethal
5	Rem per year	allowed as being without damage.
0.5	Rem	allowed per nine-month pregnancy

And what do you get from the environment?

0.05 Rem	Yearly exposure from cosmic things - the sun and all.
0.04 Rem	Additional if you live in a concrete house, due to Ra, Rd, K, and Th.
0.005 Rem	Chest X-rays, one second exposure to a source of about 200,000 Rem per year.
0.001 Rem	Watching TV, 5 hrs per day exposure to a source of about 0.005 Rem per year.

And yet there are areas on earth, people living near Thorium deposits in Brazil, and near radioactive sands in India, where the average citizen is exposed to 10 or more Rem per year, and there is no evidence of birth defects or health problems.

Thus, the exposure over the course of one year to:

A few hundred Rem per year	Would probably kill you.
10 Rem per year	Might assure you of cancer or of premature death (the real value is extremely controversial).
0.1 Rem per year	Is inescapable (environmental).

And reports from Moscow gave the following levels surrounding the tragedy at Chernobyl:

10,000 Rem per year	(1 day after, 6 miles away)
10 Rem per year	(1 week after, in Kiev)
50 Rem per year	(2 weeks after, 36 miles away)

which shows how difficult it is to estimate the long term effects from the tragedy at Chernobyl. The human damage reflects the length of time of exposure as well as the intensity of the radiation itself, and not only are some of the radioisotopes decaying (levels dropping) but many people have been moved from the most intense activity (shortened exposure). And, as mentioned, the magnitude of the accumulated exposure that is certain to cause damage to someone, has never been established.

BIOTRANSFORMATION

Biotransformation is the current term for the age-old concept of metabolism. Under either name, it represents the chemical changes that a drug undergoes, in the course of passing through the body. A third term that is occasionally encountered is detoxification. This implies that a drug is made less toxic by the body, which is not necessarily true. The term should not be used.

Biotransformation may indeed decrease the toxicity of a drug, making it less effective. But it may well increase the toxicity or potency of a drug. Some of the early sulfanilamides had to be consumed with large quantities of water, as their metabolites were of very low solubility and had been known to crystallize in the kidney tissue upon being concentrated there. Some chemicals become effective only through metabolic conversion in the body. These are currently called prodrugs.

Biotransformation can be a mechanism for specific organ delivery. An active form may not be able to enter a target organ (such as the brain with its protection of the blood-brain barrier) but a prodrug may be able to pass through this barrier and be converted (metabolized) after passage to an active form. In this case, the very barrier that would have kept it out can now serve to keep the active form in, thus decreasing the dosage needed.

Biotransformation is a process that is usually effective in making a compound more easily excreted. This is generally achieved by increasing water solubility.

The liver is by far the most important organ for metabolism, although much metabolism occurs in the lung, in blood, in the brain; metabolic enzymes can be found in almost all the tissues of the body. Almost all of the principal reactions known in organic chemistry are encountered in a telling of the metabolism story. Some of these reactions are achieved by subtle and rather specific enzymes, and others by general purpose enzymes with no specificity to speak of.

Also, there is a great deal of species variability in the route of metabolism that a specific drug might take. Not only may there be quantitative differences in the metabolic profile, but there may be examples of metabolites that are unique to a certain animal species.

OXIDATION:

The oxidation of a xenobiotic (xenos, meaning a guest or a foreigner; bios meaning life), thus something (a compound) from outside the normal living organism, usually involves the insertion of an atom of oxygen. All things being considered, this adds polarity to the molecule, and often a new functionality that can, in turn, allow an additional form of further metabolism.

Hydroxylation (or oxygen insertion at carbon). This process is widely encountered. The aromatic ring (in a hydrocarbon such as benzene) is directly attacked metabolically, to give the oxygen analog, phenol. This may in turn be conjugated (see below) or further oxygenated. Also, in the aromatic area, amphetamine (at least in some species) is oxidized to para- hydroxy amphetamine. The methyl ether of this metabolite (para- methoxyamphetamine) is a known centrally active intoxicant, and it was once suggested that the remarkable "amphetamine psychosis" might be due to the metabolism of amphetamine to the 4-hydroxy analog, and its methylation to produce this psychotropic drug in vivo. This process does not, in fact, occur.

The hydroxylation of aromatic compounds proceeds through the logical intermediate of an unconjugated intermediate, an arene- epoxide. This can be hydrolyzed and dehydrated to form the phenolic products reported. But these intermediates can also react with other nucleophiles (mercaptans) and nitrogen things, to form weird and potentially toxic intermediates. The polycyclic aromatic hydrocarbons (mutagens, carcinogenic agents) are believed to invoke their cytotoxic effects via such reactive intermediates.

The hydroxylation of aliphatic hydrocarbons is also very common. The paraffin group is an extraordinarily lipophilic thing (lipos, fat; philos, loving), very loathe to clear through the aqueous processes of the kidney. The simple insertion of an oxygen atom creates an alcohol, not only more polar by far, but also an opening wedge to further chemistry. Many drugs that carry aliphatic chains (THC, the barbiturates) are hydroxylated on this chain, with a high positional and stereospecific exactness.

A major enzyme responsible for this oxidation is commonly called cytochrome P-450. This is an enzyme containing heme, and thus ferric iron. It effects a one-electron operation (ferric to ferrous) and gets its name from the absorption at 450 nm of the carbon monoxide complex seen correlate with its action. In common language this has also been called the mixed function oxidase, since one atom of the oxygen molecule becomes attached to the substrate of the enzyme, and the other oxygen atom appears as water.

S-oxidation. Again, as with oxidation at the carbon atom, the addition of an oxygen to a sulfur atom (as in a disulfide) converts a sulfide (quite lipophilic) to a sulfoxide (very polar and also optically active, a new chiral center). Some cyclic heterocyclics such as chlorpromazine have S-oxidation to sulfoxide as a major pathway. Examples are known with further oxidation to the yet more polar sulfones.

N-oxidation is a parallel process seen with some tertiary amines, the formation of N-oxides. This procedure again adds polarity and thus water-solubility, but also has been invoked as a preliminary intermediate to N-dealkylation. An example is the metabolism of imipramine to imipramine N-oxide.

P-oxidation is known, such as the conversion of triethyl phosphine to the corresponding phosphate.

DEALKYLATION:

N-Dealkylation. The loss of a methyl group from the nitrogen atom of a base, is one of the most frequently encountered metabolic conversions. The net reaction is the conversion of a tertiary amine to a secondary one (thus generating an NH group for further metabolism) with the formation of a mole of formaldehyde. The intermediate to the dealkylation is probably a species known as an iminium ion, the consequence of the oxidative removal of a hydrogen from the alpha carbon, and the subsequent hydrolysis of the resulting Schiff base. The formaldehyde is disposed of, eventually, as carbon dioxide through the one-carbon pool.

O-Dealkylation. A similar reaction can occur with methyl ethers, but here the mechanism is probably one of direct oxidation of the methoxyl group to a formate ester, and then loss of the carbon atom as formate. Again, eventually, as carbon dioxide.

S-Dealkylation. The oxidative loss of a methyl group from sulfur is more rare, but several compounds are known in which the methyl group is transferred to another molecule enzymatically. Here, the reaction is called S-methyl transferring, and it can serve as a needed reaction in the formation of methylated biologicals. The methyl group on the sulfur of methionine is an important source of this process, using an exzyme SAM (S-adenosyl-methionine) transferase as the synthetic catalyst.

DEAMINATION:

The loss of an amine group, usually but not always in an oxidative manner, is a major procedure for the metabolism of amines. The best studied enzyme process is the monoamine oxidase system (MAO) which converts monobasic drugs (primary, secondary or tertiary amines) to the corresponding nitrogen-free acids or ketones. Some drugs (mescaline) or biologicals (histidine) with a second basic group in the molecule employ a diamine oxidase system (DAO). The inhibition of these systems (such as the monoamine oxidase inhibitors, MAOI) result in a protracted action of the otherwise metabolized drugs.

REDUCTION:

This is not a common process, but it is well established. Aldehyde groups, for example, cannot hydrate easily for excretion, but upon reduction to the corresponding alcohol, can be conjugated. Double bonds may be oxidized directly, but they are known to be hydrogenated in some compounds. A major documentation of reduction is seen in the organic nitro group, itself rather non-polar and without sites of conjugation, but upon reduction to the hydroxylamine, or to the amine itself, eminently excretable.

HYDROLYSIS:

The art of the removal of water from polar things such as acids and bases (to

form amides) or acids and alcohols (to form esters) as a manner of reducing polarity, has its direct opposite metabolically, in that these relatively non-polar products can be hydrolyzed back to their highly polar components. The serum esterase system (acetylcholine esterase) has already been mentioned in conjunction with the mechanism of the cholinergic synapse operation. But there are the so-called pseudo-esterases, that are completely indiscriminate as to just what esters they go after. Most esters (such as cocaine) and amides (such as phenacetin) are readily hydrolyzed *in vivo*.

CONJUGATION:

The term "conjugation" has several meanings in the dictionary (demonstrations of verb inflections, fusion of gametes instead of fertilization, the transfer of DNA, etc.) none of which apply to chemistry or pharmacology. In chemistry, this term refers to the interactions of alternate double bonds, allowing a low energy repository for a tired electron. In pharmacology, the term refers to the covalent coupling of a new chemical onto a drug, to greatly increase its water solubility.

The most frequently seen agent is glucuronic acid. This is a molecule of glucose in which the 6-carbon hydroxyl function has been oxidized to a carboxyl group (the corresponding oxidation of the 1-carbon atom yields gluconic acid or its lactone, gluconolactone). Glucuronic acid interacts with many active hydrogen intermediates to form esters, amides, sulfides, and on and on, in which the alpha carbon is coupled directly to the hetero-atom. The "up" orientation shown is called a "beta" glucoronide, the down (and more rare, biologically) the alpha glucoronide. These are not esters or amides through the carboxylic acid function; these are glycosides through the "aldehyde" carbon of glucose. The effectiveness of the glucoronides in water solubilization of drugs is due to the presence of three new hydroxyl groups, and a strong acid function (the carboxylic acid group) that can form a highly ionized salt.

The second most frequently encountered conjugating material is sulfuric acid. Alcohols, which are only very weak acids at best, can and do form mono-esters with sulfuric acid to form mono-alkyl esters of sulfuric acid. The second acid function of the sulfuric acid is still a very strong acid, and is completely free in the body. Thus, it forms a salt (statistically the sodium salt) which is highly ionic and thus easily cleared from the body. A third conjugating agent is glycine, the simplest amino acid. It can be found often as an amide with a carboxyl group (benzoic acid to hippuric acid) yielding an acid of similar strength, but one with an additional inventory of polar functions (an added amide link).

ENZYMES:

As virtually all biotransformational reactions are mediated by enzymes, some comment on them may be useful here. Enzymes are great big proteinacious things that catalyze, usually highly selectively, some particular reaction. Recently there has been an effort to categorize them systematically. And the results are terms such as E.C. 5.3.3.1. Let me unravel this as best I can. This is quite arbitrary since many

mechanisms are unknown, and further, many enzymatic reactions involve several transformations, any one of which could be considered as being of highest priority. The "simple" explanations of this code are so complex, that I will try to simply outline the hopes of the writers!

E.C. stands for the Enzyme Commission who put this nomenclature all together.

The first digit generally stands for the class or division of the enzyme's action (there are six) and all following numbers are sub-classes or sub-sub-classes of these divisions. In almost all examples, the last number is the tally of examples in the class being defined.

E.C.1. are oxidoreductases.

In the older nomenclature, these are the dehydrogenases, but since for every oxidation, there is a reduction, there are here also the reductases.

The second number represents the hydrogen donor, i.e., 1 is alcohol, 2 is carbonyl, etc.

The third number represent the acceptor involved, i.e., 1 is NAD(P), 2 is cytochrome, three is molecular oxygen, 4 is a disulfide, 5 is a quinone, etc.

E.C.2. are transferases.

They have a history of being referred to by the group being transferred (aminotransferase, methyltranferase) but sometimes the breaking of a bond gives the image of a thing being cleaved (as with phosphorase). And more uncertainty is involved in transferases where there is electron transfer as well (as in the transaminases) in that they can be considered as an oxidoreductase as well.

The second number indicates the identity of the group being transferred, i.e., 1 is a one-carbon group, 2 is an aldehyde or ketone group, and 3 is a glycosyl group, etc.

The third number here subdivides the items in the second group; thus with E.C.1. (the one-carbon group), 1 is a methyl, 2 is a hydroxymethyltranferase, etc.

E.C.3. are hydrolysases.

They are hydrolytic enzymes that cleave (largely) the C-O, the C-N and the C-C bond. The potential confusion with transferases is mentioned above. Additional complications come from the fact that substrates are not always specific, and that (in the case of some esterolytic proteases) some synthetic substrates are more acceptable substrates than natural peptide bonds. The early history of enzyme studies has dictated much in this classification scheme.

The second number is the bond hydrolyzed; 1 is an ester, 2 is a glycosidase, etc.

The third number is the nature of the substrate, as for example with the esterases, 1 is a carboxylic acid ester, 2 is a thiol ester, and 3 is a phosphoric monoester. With other substrates these numbers are casually reassigned.

E.C.4. are lysases.

These are enzymes that cleave the C-O, the C-N or the C-C bonds by elimination, leaving double bonds, or conversely, adding groups to double bonds. But in whatever reaction occurs, there should be some intermediate of a double bond.

The second figure is a code for the bond broken; 1 for C-C, 2 for C-O etc.

The third figure gives further information on the group eliminated, i.e., CO2 or H2O.

E.C.5. are isomerases.

These enzymes catalyse geometric or structural changes within a molecule. There are (in older nomenclature) the racemases, epimerases, isomerases, mutases, etc. This isomerization can be brought about by oxidation-reduction reactions but if the donor and acceptor are in the same molecule they are classified here even if NAD(P) is involved.

The second and third figures are assigned according to the type of isomerization and the type of substrate.

E.C.6. are ligases (or synthetases).

These are enzymes that join together two separate molecules, coupled with the hydrolysis of a high energy phosphate bond. Sometimes a specific group name is involved, i.e. carboxylase.

The second figure codes the bond formed; 1 for C-O bonds (acylation of RNA), 2 for C-S bonds (acyl CoA derivatives), etc.

The third figure is used only with C-N ligases.

See, the chemistry of enzymes is really quite simple — it is the organization that is hopelessly complex.

PHARMACOKINETICS AND PHARMACODYNAMICS:

Pharmacokinetics is the study of the motion of a drug into, about, through, and out of a body. Pharmacodynamics is the study of the action of a drug within the body, on all systems from the molecular to the organ.

Consider three steps in the action of a drug:

Dose given $\xleftarrow{\text{PK}}$ Tissue concentration $\xleftarrow{\text{PD}}$ Effect achieved

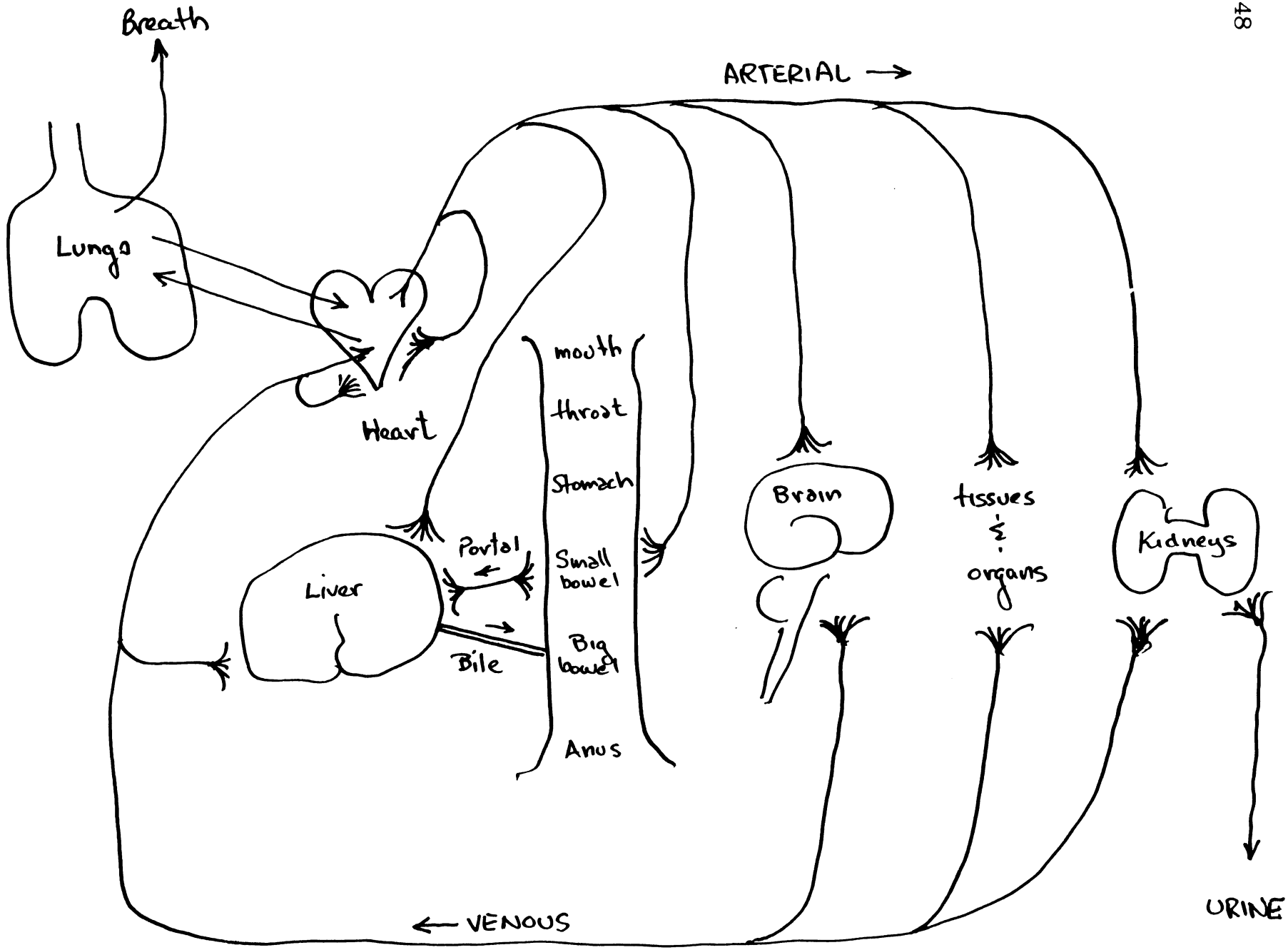
pharmacokinetics (PK) is the mechanical distribution following administration up to the actual localization as some site of action, and pharmacodynamicis (PD) is the chronology and duration of the drug's action.

Routes of administration:

Quite obviously, for a drug to have an effect on the body or on something within the body, it must get inside somehow. Hence, there must be a route of administration. And once inside, it is usually carried to its target site by the blood. On the following sheet. there is a highly stylized schematic of the body showing the blood flow.

It is easier to visualize the administration process if you were to consider the G.I. tract as being similar to the opening left in a cored apple. The body of the apple represents the body of the person, and the hole in the apple represents the long tube through the body from mouth to throat to stomach to small bowel to large bowel to rectum to anus. Although this tube is physically within the body, it is both embryonically and functionally on the outside. In other words, something can be eaten, and pass all the way through to final excretion in the stools, and from the pharmacological point of view never have been in the body at all. Of course the effects of its passage may be noted, as it may be metabolized by the intestinal flora or it may exude some toxin, but these products themselves must (as a rule) be absorbed from this tube to be effective.

A major route of administration is by mouth, and this process is called "oral" or p.o. (for per os, or by the mouth; but be careful with this noun, as "os" in latin also means bone). And absorption into the body can occur at any point along the length of this tube. When a drug is taken into the mouth and held there, absorption can occur beneath the tongue where the tissues separating the mouth from the blood are extremely thin (sublingual) or from the cheek or mouth itself (buccal). When swallowed, the absorption can occur from the stomach (gastric) or the small bowel (intestinal). And absorption from the rectum can occur very easily (rectal). In all of these situations (except intestinal) the absorption is directly into the blood stream and as it does not involve the intestines, can be called parenteral (avoiding the intestines).



CIRCULATION OF BLOOD IN THE BODY

When absorption occurs from the small bowel (enteral absorption) it passes not into the venous blood directly, but into a capillary system that starts from the mesentery proper and ends in a capillary system that is attached to the liver. This process is called the hepatic portal, and is neither an artery nor a vein, as it operates without help from the heart (hepatic, pertaining to the liver; portal, going from one capillary bed to another).

There are many other parenteral routes for drug administration, each with its advantages and its disadvantages. One of the most frequently employed is injection with a syringe and needle. The drug is inserted within the substance of a muscle (intramuscular, i.m.), within a vein (intravenous, i.v.), within an artery (intraarterial), or under the skin (subcutaneous, s.c.). Or without the use of needles, there may be absorption through the skin (transdermal or percutaneous), by inhalation (absorption directly from the lungs), or via the mucosa of the nasal septum (insufflation, or snorting). In general, the parenteral routes involving injection eliminate loss from vomiting, provide a relatively fixed dose at an accurately determined time, since there are no uncertainties of absorption. But then they are also routes, in that once employed, the drug will remain within the body without the possibility of recall. Also, to a large measure, these routes by-pass the liver at least on the first pass, and so provide the least opportunity for metabolism prior to reaching the target site. Inhalation, and especially arterial injection employing the carotid, provide the most rapid access to the brain, with a minimum of dilution with venous blood. But inhalation requires a misting or vaporization of the drug, and a certain amount always trickles down the throat, so the net dosages is uncertain; and arterial puncture carries a real risk of shock.

Volume of Distribution:

If the blood were a single fluid isolated from any other tissue, then a given dose of drug administered to the blood would be distributed just that far and no further. The blood concentration would be the dose given divided by the volume of the blood. In truth, drugs go out of the blood into tissue, into intercellular spaces, into the cells, and sometimes this is an active process that seems to effectively deplete the blood of drug. The blood concentration is lower than calculated from dose and blood inventory. The apparent volume of this diffusion, in other words, how much "blood" would there have to have been to account for the low blood concentration, is called the volume of distribution. This is a measure of the extent of tissue delivery of a drug, and reflects its lipophilicity.

Transportation:

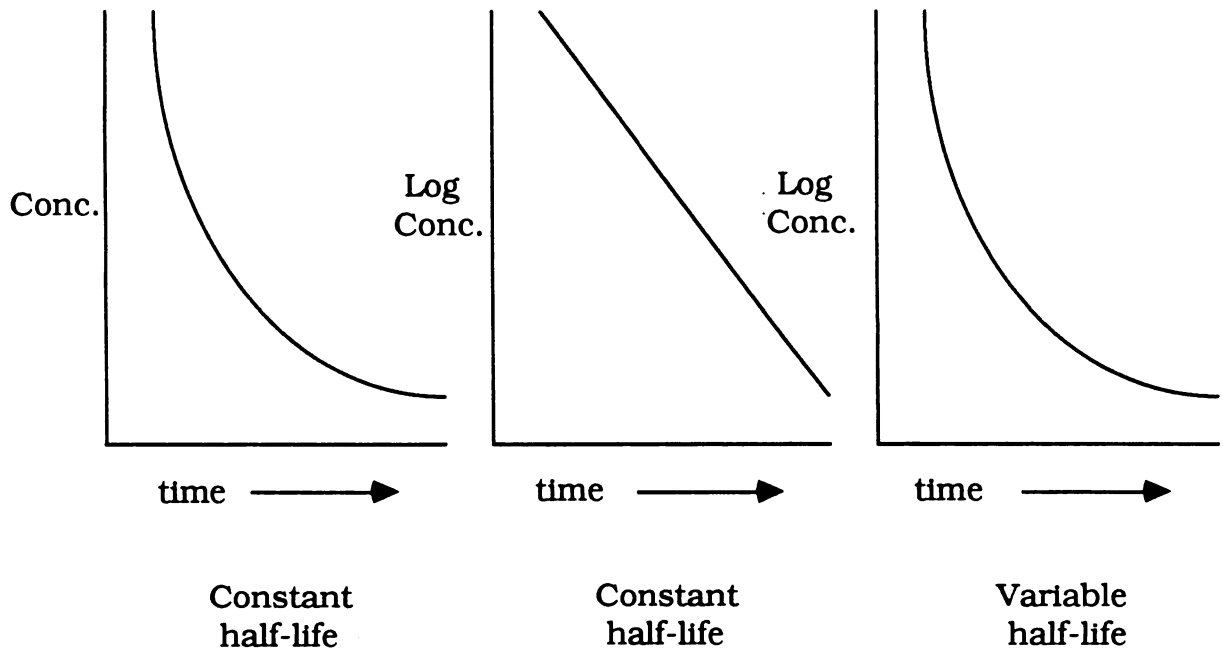
The circulating blood is by far the most important mode of transportation. The actual kinetics will be discussed below, but in general there is an immediate maximum achieved following i.v. injection, with longer delayed maxima following i.m. and finally oral administration. The concept "volume of distribution" is a term frequently used to demonstrate the degree that the drug has been partitioned out of the blood and into tissue. This term is a measure of the effective volume through which the drug has been

distributed, knowing the plasma concentration and the amount of drug injected. Of course, if the administered sample were contained solely in the blood, the volume of distribution would be the actual volume of blood in the body, but very often, especially with drugs that are highly lipophylic, the actual plasma concentration is surprisingly low (large volume of distribution) since there is an extraction of the drug into tissue or fat stores. This brings up the concept of bio- availability, a measure of the quantity of drug that is actually free to be effective at the expected site of action. If the target organ is the brain, and most of the drug administered is sequestered into fat, then the availability will be low, and the duration of action or detectability (availability, as it partitions out of fat again and into the blood) might well be long.

The blood also serves as a transport of the drug to the kidneys, the usual major route of excretion. As the blood is filtered to form urine, there can be extensive reabsorption from the kidneys, often depending heavily on the urinary pH. Again this is a matter of partitioning. If a drug is a strong base (let us take amphetamine as an example) then with acidic urine the drug will be considerably ionized, and will partition preferentially to the aqueous (be excreted). And with basic urine, the drug will be relatively less ionized, and will have an easier penetration of tissue, thus be more completely reabsorbed (be conserved). In test subjects, amphetamine administered with sustained dosages of ammonium chloride will be 50% excreted in the first 24 hours; amphetamine administered with sustained dosages of sodium acetate will be 3% excreted in 24 hours.

Plasma half-life:

The concept of half-life is probably first introduced from the understanding of radioactive decay. If there are 100 units of something at a given time, and it disappears



at a rate proportionate to the amount present, then the amount of that something will be reduced to half of the original value in a given unit of time, and will be half of the remaining value in one more such unit of time, and to one half of the remaining etc etc etc. That unit of time is the half life of the something. When this system is plotted with a linear measure of amount on the X-axis, there is a sloping curve with an asymptotic approach to the Y-axis. On a logarithmic Y-axis presentation, the curve is a straight line. The decrease of the blood concentration of a drug usually follows something of the same nature. This is a measure of loss by dilution, and if the kidney clears something at a rate proportional to its concentration, there will be a linear logarithmic relationship. In reality, the actual plot is something like the third example shown, in that there is a gradual increase in apparent half-life with time. This is due to the complications suggested above, that there is a partition into areas that are less readily accessible, and that they are a delayed source of drug. This non-linear presentation thus defines a multi-compartment model, with each compartment having separate and individual kinetics.

Metabolism:

The role of metabolism in the disposition of a drug has already been discussed. It must be understood that often the apparent serum concentration of a drug is not the measured quantity as determined by isolation and analysis, but the measure of some property that may not distinguish between the parent drug and its metabolites. Examples are the assay of radioactivity of a labeled drug, or the antigen-antibody response in a radioimmune assay. The non-linearity of an apparent clearance curve (the half-life curve above) may be due to a change of composition of the species being measured.

Activity correlation with serum level:

The relationship between the amount of a drug present in the body and the activity of a drug, is the essence of pharmacodynamics. It would be tidy to think that when the serum level gets to an "active" value, the drug becomes effective, and when it drops below this level, it ceases being active. Things don't work that way.

The serum levels are not accurate measures of active levels, since the active sites of action are rarely in the blood. Several aspects must be considered in relating levels and actions.

There is a touch of the dy/dt in drug action. In some drug responses, it is the rate of change of the concentration, even more than the concentration itself that determines the expression of action. A rapidly changing serum concentration evokes a response, often independent of the absolute concentration. This is a justification (or rationalization) for intravenous administration, where the rate of change in blood concentration is the greatest. And if the action of the drug occurs at some site behind a barrier of tissue (getting into an organ) or a barrier of polar exclusion (the blood-brain barrier, getting into the brain) then there may be partition delays in going from blood to receptor site. If metabolism is required to generate the intrinsically active species,

then repeated exposure to metabolic enzymes (in the liver) may be needed before action is realized.

There is the concept of tolerance to be considered. The loss of response to a drug that follows repeated exposure is called tolerance (the loss itself is referred to as becoming refractory to a drug). This effect is apparent with many drugs following day after day exposure, although it may be observed sometimes only for certain properties of the drug and not for others. But there is a short-term refraction that can be seen during the course of single, acute exposure. It is a sort of tiredness of the receptor in responding, a depletion of things released to effect action, a saturation of some feature that can take no more stimulation. A close companion of this tolerance is the often seen behavior of increasing the dosage to maintain the effect. Although a common feature in para-medical drug use, it is also frequently seen in self-medication with prescription drugs. "One didn't do it anymore, so I took two."

The subdivisions of tolerance have recently been organized into categories of disposition, function, and behavioral. Disposition tolerance deals with changes, upon repeated exposure, of absorption, distribution (within the body), metabolism, excretion, and generally the pharmacodynamics of the given drug. Functional tolerance deals with changes following repeated usage, in receptors (their number and identities), secondary neurotransmitter populations, feedback function at the neuropharmacological level, tissue change, and changes in response to challenging agonists and antagonists. Behavioral tolerance is a measure of displayed action as a function of repeated dosages.

There is the concept of dependence. Here one easily opens a hornet's nest. For years the medical community was saddled with the pejorative word "addiction." When addiction drifted from the medical world into the legal world, when it ceased to be a health problem and became, rather, a crime, there was an effective move made to discard it. It is now largely replaced with the term "dependence", subdivided into the classes of psychological dependence, and physical dependence. Psychological dependence is everywhere, with the explanation of it lying more with the psychologists than with the pharmacologists. We like jellybeans and really don't feel right without having them available when wanted. The body has not really modified its internal biochemistry to handle jellybeans in an unusual manner, so that the abrupt withdrawal of a person from jellybeans will probably not invoke a life-threatening crisis. This is a major shaper and former of our daily behavior patterns. In physical dependence there is something deeper and more subtle that has taken place within the body. Constant exposure to a barbiturate results in an enzymatic induction that modifies a person's response to the barbiturate. The body has come to depend on that drug for "normal" behavior. In some cases, with some drugs, there may have been the induction of neurological changes that may be only slowly reversed. But in most cases, the chemistry is not known, but an abrupt withdrawal of a person from his "barbiturate" may invoke a truly life-threatening crisis.

The action of a drug can be quite different from that expected, on occasion, due to the presence of a second substance.

If this second substance is itself without pharmacological action, but it decreases the expected action of an administered drug, it is called an inhibitor or antagonist. It may fit nicely into the receptor site (the lock and key theory) but not do anything, but it keeps the desired agonist out. (A drug that keeps another drug from being active is an antagonist; a drug that achieves the action of another drug is called an agonist [in literature it would be the protagonist]; a drug that is the normal and expected agonist is called the substrate).

If this second substance is itself without pharmacological action, but it increases the expected action of an administered drug, it is called a potentiator. Sometimes some unusual body chemistry may unexpectedly promote a modest drug into a highly potent drug, with unexpected responses (remember tachyphylaxis in lecture #3?) such as an idiosyncratic hypersensitivity (idio- is personal; syn- is together; krasis, to mingle – something that is a person's own peculiar character). One person in a thousand has a very threatening response to penicillin. Somewhere, something is potentiating the body's response to this drug.

If this second substance is itself with pharmacological action, and its action plus that of the action of the administered drug produces an effect that is more than the expected sum of the two, there is seen the phenomenon of synergism. Simply stated, 2+2=5. If I come across a comatose patient with a modest amount of a barbiturate present (the current medical lingo is "on board,") and there is no reasonable connection between the barb level and the clinical picture, I will automatically run a blood alcohol. A modest (and non- threatening) blood barbiturate level, combined with a modest (and non-worrisome) blood alcohol level, will be found quite regularly in a person who has died unexpectedly. In this case, dwell for a minute on the subtleties of the legal ramifications.

The cause of death is clear. A modest barb level, a modest alcohol level, with a synergistic (and unintended?) interaction that proved fatal. The booze may have made the sleeping pill unneeded, but it was part of the pattern of retiring, and there was some uncertainty as to whether the first secobarb had been indeed taken. Inadvertent overdose? Attempted suicide? A $500,000 insurance policy may ride on the wording of your report.

Polypharmacy:

All too often, there is the presence of several drugs in a patient at the same time. Usually, the administering physician of some of these is unaware of the presence of others of these. Sometimes there is a multitude of simultaneous crises that must all be treated and the ends justify the means. But in almost every case, one may say with assurance that the particular combination of drugs present in the patient has never been tested before in any animal under any experimental condition. The possibilities of all of the above drug interactions are real, and even to be expected. A report appeared about ten years ago, that surveyed the drug inventory "on board" in a typical patient in ICU (intensive care unit) in a hospital in New York. The average tally was

sixteen. In fairness, there was a dedicated effort to treat every life-threatening twinge with some anti-twinge medicine, and many things were being used at the same time. But in fact, this extraordinary combination represented, in each patient, a novel and unprecedented experiment of polypharmacy with all the inhibitions, potentiations and synergisms imaginable. I am amazed that we so often survive our trauma.

PARTITION, SOLUBILITY, ION-PAIRS:

In the next two lectures, I want to talk about the principles involved in the isolation of drugs from mixtures such as body fluids, and the procedures that are currently in use to evaluate this isolation.

There are two aspects to drug analysis, and they will be discussed separately. How does one achieve a separation, and how does one know what has been achieved. Lecture #7 is directed to the separation techniques: the seeability will be assumed for the moment.

Partition:

The concept of the partition coefficient is fundamental to every aspect of drug isolation, of purification, and of chromatographic analysis of both mixtures and of isolates.

The partition coefficient is the ratio of concentrations found when a relatively small amount of a material is distributed between two immiscible liquids. Let me start by defining some terms:

Miscible: Literally, capable of being mixed. In the analytical jargon, a bit more restricted – (liquids) capable of being mixed in any proportion without separation into two phases. This means, regardless of the relative amounts admixed, there is a single phase remaining, after mixing. Thus, the term "miscible in all proportions" is redundant; it is the same as "miscible."

Immiscible: Two liquids are immiscible when they separate into two phases following intimate mixing. Some points should be emphasized. Consider the consequences to mixing equal volumes of methylene chloride and 0.1 N HCl. Two phases will separate after standing for a bit.

Specific Gravity: This is measure of how heavy a given volume of liquid is. The common term is "density" which requires dimensions – so and so many grams per liter, or pounds per cubic foot. Specific gravity is a ration to that of water, which is approximately 1.0 The importance of this term in extraction work, is that when two solvents are immiscible, and of sufficiently different specific gravities, the more dense one will be one the bottom. A typical range of specific gravities for common solvents are: hexane 0.66, ether 0.70, ethyl acetate 0.90, water 1.00, and methylene chloride 1.32. This last solvent, called dichlor or CH_2Cl_2, is commonly used and usually on the bottom, unless there is a lot of salt or other dissolved stuff in the water phase.

One or both of these phases might be cloudy. This is the result of a fine suspension of droplets of one liquid in the other. This will interfere with both accuracy

and with spectroscopic measurement. The only practical way to resolve this source of error is to allow the system to stand until the phases are clear, or to centrifuge the mixture until clear.

Although the volumes before mixing were equal, they will be different afterwards. The solubility of dilute HCl in CH_2Cl_2 will be different that the solubility of CH_2Cl_2 in dilute HCl. Therefore one liquid dilutes the other more than the other diluting the first.

And the identities of the two liquids are now different. The CH_2Cl_2 is no longer CH_2Cl_2, but is now CH_2Cl_2 saturated with dilute HCl, and visa versa. Also remember, that solubilities are always temperature dependent. Thus the composition of these two separated phases will depend on the temperature.

A last point. As said above, everything is soluble to some extent in everything else. Thus, if a sufficiently small amount of one liquid is added to the second, one phase may result even though the liquids are immiscible.

Relatively small amount of material: An amount of substance (the material to have its partition coefficient determined) must be small for two reasons: (1) It must be soluble in either phase alone, and (2) it cannot appreciably contribute to the composition of the solvent.

Concentrations: Clearly if unequal volumes of immiscible solvents are used in the determination of a partition coefficient, the solvent in the larger amount will extract a disproportionate amount of test material. The measure of concentrations obviates the need of controlling volumes. And since the ratio alone is used the express the coefficient, there is no reason to know exactly the quantity of the test material employed. The coefficient is without dimension, but does require direction. The partition between A and B is such-and-such. The value between B and A would be the reciprocal of such-and-such.

Solubility:

The concept of solubility has often gotten into the discussion of partition, and it doesn't really belong there. Everything is soluble to some extent in everything else, so there is no predictability as to partition from this property per se. Solubility is simply the quantity of a material (solid, liquid, or gas) that will dissolve in a second material (usually a liquid). The material going into solution is called the solute, the second material is called the solvent, and the consequence is called the solution. Most solutes have a positive coefficient of solubility; the warmer the solvent, the greater the solubility. Solutes can vary enormously in their rates of dissolving. This can relate to crystal size and to effectiveness of agitation. The end concentration should be independent of rate.

Some compounds have several crystal forms (called polymorphs, from poly- many; and morph- shapes or forms). A true polymorph can have a different rate of

solution as well as dissimilar properties such as solid spectra, color, vapor pressure, etc. All forms once in solution are identical.

The actual degree of solubility is dictated by many properties. The most important is the topic of polarity. The more polar a solute, the more the solubility in a polar solvent, and visa versa. Here one finds the terms lipophilicity and hydrophilicity as well.

Polarity:

This term deals with the effective separation of opposites, combined with the association of similars.

With the concept of the separation of opposites, the major measure is the electrical charges that can be located within a molecule, and the distance between these locations. The greater the charge or the greater the separation, the greater the polarity. The distance of separations usually deal with atoms in a molecule or in a crystal, and are usually quite limited in variation. But the intensity of charges can be extremely variable. The union of this separation and these charges is called the dielectric constant.

The charges can be full plus and minus ions. With solutes, an excellent example is NaCl, where the Na(+) is over here and the Cl(-) is over there. There can be no more charge identity nor more charge separation. Many inorganic salts have a high ionic character, and to that extent, they are soluble in polar solvents. Similarly, the salts of many organics are essentially completely ionized, either the acid salts of amines, or the base salts of carboxylic acids. These, too, are highly polar, and soluble in polar solvents. With solvents, one cannot find examples of completely ionized liquids easily, since high ionization encourages charge separation, organization, and thus crystallization. But there are quite a few solvents that are almost ionized. Water is fantastic, since it has a negative oxygen that sits like an elbow between two positive hydrogen atoms, and so presents an extremely effective dipole. The lower alcohols have the same bend. Solvents with dative bonds — bonds involving completely donated electrons such as the N-O or the S-O bond in amine oxides or sulfoxides, are also amongst the most polar. Dimethylsulfoxide is an example.

With the charges something less than full + and -, the polarity is compromised but still there. With solutes, there are molecules without an overt hydroxyl or amino group with the corresponding active hydrogens, but there may still be an oxygen function such as a carbonyl group (esters, ketones or aldehydes) or a nitro group or an ether function. Here there are the assignments of the magic lower-case Greek ∂^+ and ∂^-, indicating that there is a separation of partial charges towards separate locations within the molecule. The dipole moment is not large, but it is very real. Solvents can show the same intermediate compromise. Esters (ethyl formate, methyl acetate), carbonyl compounds (acetone, cyclohexanone), nitromethane, anisole are examples of the "half-way" charge separation.

COMPARATIVE POLARITIES OF SEVERAL SOLVENTS

Eo (Al2O3)		
	-0.25	fluoroalkanes
-0.20		
-0.10		
0.00	0.00	pentane, hexane, isooctane, pet-ether
	0.04	cyclohexane, n-decane
	0.05	cyclopentane
0.10		
	0.15	carbon disulfide
	0.18	carbon tetrachloride
0.20		
	0.26	xylene
	0.28	isopropyl ether
0.30	0.29	toluene
	0.32	benzene
	0.38	diethyl ether
0.40	0.40	chloroform
	0.42	methylene chloride
	0.45	tetrahydrofuran
0.50	0.50	ethylene chloride, methylethyl ketone
	0.56	acetone, dioxane
	0.58	ethyl acetate
0.60		
	0.62	dimethylsulfoxide, aniline, diethylamine
	0.64	nitromethane, acetonitrile
0.70	0.71	pyridine
0.80	0.82	propanol, isopropanol
	0.88	ethanol
0.90		
	0.95	methanol
1.00		
1.10	1.11	ethyleneglycol
	big	acetic acid
	very big	water

And the effective charge can be extremely small, still giving some dipole, but now allowing "non-bonding" interactions to have some influence. The chloro-carbon bond is a good example. As a solute, the presence of aliphatic halides will clearly aid in the solubilization of drugs (DDT, insecticides generally, TCDD) into solvents that have similar atoms for interaction. Here one finds butyl chloride, methylene chloride, carbon tetrachloride, and some of the more polar freons. The nature and the number of the halides both contribute to the non- bonding aspect of the association.

With totally non-ionic, non-charge separated materials, it is this non-bonding phenomenon that allows solubility, for it is only the "non-bonding" nature of the solute and the solvent that brings things together. They don't dissolve actively as much as they dissolve by default. Things that are aliphatic, with little polar aspect, will prefer to dissolve in aliphatic solvents. Hydrocarbons dissolve hydrocarbons.

And strangely, there is an aspect of polarity that is substantially anti-bonding. When the hydrogen of the hydrocarbon is replaced with the fluorine of the fluorocarbon, one moves yet further into the camp of the anti-bonding and the repulsive interaction. This atomic repulsion in the perfluorohydrocarbons makes it an unattractive solvent for even the simple hydrocarbons. Perfluorohexane is immiscible with hexane.

Hydrophilicity:

The relative degree of attraction to water. Things that are hydrophilic (hydro- water; philic- loving) bind closely to water. They tend to avoid fats, to associate with surfaces and systems that are highly hydrated, and to be soluble in polar solvents. The term lipophobic (lipo- fat; phobia- fear) can be used interchangeably.

Lipophilicity:

Lipophylicity, on the other hand is a measure of attraction to fat. If a material tends to partition to the organic phase from the aqueous phase, one calls it lipophilic. It is hydrophobic. Lipids were originally those components of living tissue that extracted into chloroform, but the term has now come to imply fats and glycerides. In a general usage concept now current in biology, it is accepted that compounds can enter tissue (into cells, into the brain, over to the other side of the membrane) only by leaving water and going through fat (the lipoid structures of the separating membrane). Hence, there is an equation between lipophilicity and bioavailability. But fat is not an active site of receptors, so things are not that simple.

Zwitterions:

Some compounds have an acidic and a basic group within the same molecule. A classic example is morphine. There is a phenolic OH group which is an acid, and a tertiary amine which is a base. Thus the molecule forms its own salt, and is correspondingly difficult to extract from water. It is always a salt, regardless of the pH of the water. The optimum extraction conditions are when the pH of the solvent

allows an exact internal neutralization of these molecular opposites. This is called the isoelectric point, or the pKi.

Ion-pairs:

The concept of ion-pair identity and extraction is crucial to all aspects of drug analysis. Let me present a reasonable analysis situation, that will consolidate all of the above discussions on polarity and solvent, but designed to emphasize ion-pairs.

Let us consider the partition that would be seen from a compound such as phencyclidine between dilute HCl and methylene chloride. Phencyclidine is a strong base, and forms a nearly completely ionized salt with HCl. This ion-pair will, of course, partition completely into the aqueous phase. But, on the other hand, phencyclidine has some 10 methylene groups, and as such it is a fat, and will partition completely into the organic phase. What is the net result? The amine hydrochloride wanting to drag the hydrocarbon into the aqueous, or the hydrocarbon, wanting to drag the amine salt into the organic? There will be a partition coefficient, since phencyclidine hydrochloride is quite soluble in both dilute HCl and in methylene chloride. The observed consequence of this contest is that most (maybe 95%) of the base does go into the organic phase, and if it is evaporated, one can obtain an excellent yield of the product amine hydrochloride.

The hydrochloric acid goes into the organic with the base. The amine (+) chloride (-) goes into the organic. This is an ion-pair.

Effect of pH:

Most drugs are bases. Therefore, most aqueous systems that might contain drugs (urine, blood, gastric content, tissue homogenate) will have the drug present charged or uncharged (ionic or un-ionic) depending upon the pH of the system being extracted. In general, if a drug is charged (as a base would be in an acidic medium), it will not be extracted from an aqueous environment. In general, if a base is not charged (as a base would be in an alkaline medium) it will be extracted from an aqueous medium. Thus a first approach for the isolation of any (basic) drug is to:

(1) acidify, and extract with an organic solvent, to remove both acidic and neutral contaminants; then,
(2) make basic, and extract, with an organic solvent, the drug being sought.

THE DRUG WILL BE IN THE ORGANIC PHASE

or:

(1) make basic, and extract the drug being sought as well as all neutral contaminants; then,
(2) extract the organic phase with dilute acid.

THE DRUG WILL BE IN THE AQUEOUS PHASE

Become completely familiar with this pair of actions. This is the heart of all extraction techniques. With most drug assays, employing a search for basic drugs, one of these two processes will be employed and will work. In a few of the remaining problems, the concept of ion-pairing will give difficulty in identifying the phase where the sought-after drug will be found.

This concept of adjusting the pH to direct the drug's extraction is crucial to the understanding of drug analysis. Under basic conditions, the drug is not charged, and will follow the non-polar phase. Under acidic conditions, the drug is charged and will probably follow the polar phase.

When the drug is acidic, of course, the entire pH dependency will be reversed.

Absorption and Desorption:

Although the liquid-liquid extraction built upon, above, is a major structure of drug isolation, many techniques have been developed that call upon absorption and desorption from resins of one sort or another. In these systems, the subtlety of partition has generally yielded to the all or none aspect of absorption.

Cation-exchange resins: The first of the ion-exchange resins was a cation-exchange resin, produced by the sulfonation of polystyrene beads. Visualize a million million microscopic spheres, all coated with a chemically attached sulfonic acid group (strong acid) that because of this attachment could never leave the surface of the microsphere. Now put this million million spheres into a column onto which you could pour some aqueous solution, and out of which some aqueous content would be displaced. The sulfonic group itself is absolutely fixed on the resin sphere, and so cannot be washed off. If all the sulfonic acids are indeed sulfonic acids with their hydrogen ions, and they are covalently attached to the insoluble microspheres [all resin-SO_2O^- H^+] then one could wash the column forever with water, and there would be no chemistry. Water onto the top, water out of the bottom. But if one were to introduce another cation (a Na+ ion, maybe, or a base-amine-H^+ from a urine that contained a positive cation other than H^+) then that ion would exchange with the H+ at the top of the column, releasing a little bit of HCl out the bottom.

Thus all cations, organic or inorganic, will be exchanged and absorbed, releasing an equivalent of HCl from the column.

Then, in time, after water washing, the administration of strong HCl will displace the absorbed cations (Na or drug) and an isolation from urine or blood has been effected. As an alternate procedure, the exchanged cations can be displaced with other cations in a gradient elution process, allowing a chromotagraphic separation of the cations present.

Anion-exchange resin: Similarly, the attachment of a quaternary ammonium hydroxide to these polystyrene microspheres, allows an anionic analogy of this resin.

This is called an anionic exchange resin, and indeed effectively exchanges anions from aqueous systems. In the hydroxyl form, all acidic drugs from urine can be absorbed, to be eventually displaced with either sodium hydroxide or an appropriate buffer.

It is obvious that if water is run through these two resins (the cation resin in the H^+ form and the anion resin in the OH^- form), all ions will be removed. This product is called de-ionized water, and is the 89 cent a gallon stuff you buy at Safeways for the electric iron. Alternately, the distillation of water can effectively leave the salts (as non-volatiles) behind, and this is called distilled water. Ion-exchanging removes ions, and distillation removes non-volatiles.

Another use of the cation-anion exchange resins is the replacement of cations with sodium ion, and the replacement of anions with chloride ion. Here, water is passed through these two resins (the cation resin in the Na+ form and the anion resin in the Cl- form) then all salts present will be replaced with their equivalency in NaCl. This is called soft water, and the process is called water softening. Here the "hard" ions such as Ca^{++} and Mg^{++} and CO_3^- are removed.

Neutral resins: Resins exist without any charged radicals upon them at all. One of the earliest of these was the XAD resin which was, in effect, the original polystyrene bead without treatment with either sulfonation or alkylation chemicals. It has a neutral surface, and can absorb things simply by the large amount of organic surface that it presents. If one were to take a urine specimen and adjust the pH to about 8.0, then all the drugs that might be present would be, to some extent, unionized. The bases are obviously free, and the acids present would have some statistical probability of not being completely ionic. With the passage of this urine specimen down the XAD resin, there would be some absorption of drugs present (plus the absorption of everything else that is slightly basic in urine), and subsequent elution would give a cleaned-up sample for subsequent separations.

The C-18 resin column plays the same role. Here there is a silica column that has had octadecyl groups attached to all of the acidic silica sites, and the column acts as if it were a big piece of paraffin. Again, elution with an organic solvent can effectively concentrate the drug fraction from urine.

Extraction Efficiency:

A common question that arises in drug extraction, is how often, and with how much solvent does one extract. As I had already mentioned, you wish to use the worst solvent you can, that is still adequate. This will give the smallest quantity of contamination.

But the best use of that solvent is an interesting calculation, and it depends on the partition coefficients of both the desired material, and that of the principal contaminating chemical. Below there is an example, set up as a hypothetical illustration, of the water-ether partition of two chemicals. Benzoic acid is assumed to be preferentially taken up in the ether (partition coefficient of 2.0, ether/water) and

salicylic acid the opposite (partition coefficient 0.5, ether water). With a fixed volume of water (1 L.) and of ether (1 L.) there are two demonstration of extraction, where the ether is used all in one extract, or divided into two half-quantities for two extractions. Notice that as a fixed volume of extraction solvent is used in more and more smaller and smaller portions, the recovery yield continues to rise, but the purity of the isolate begins to deteriorate.

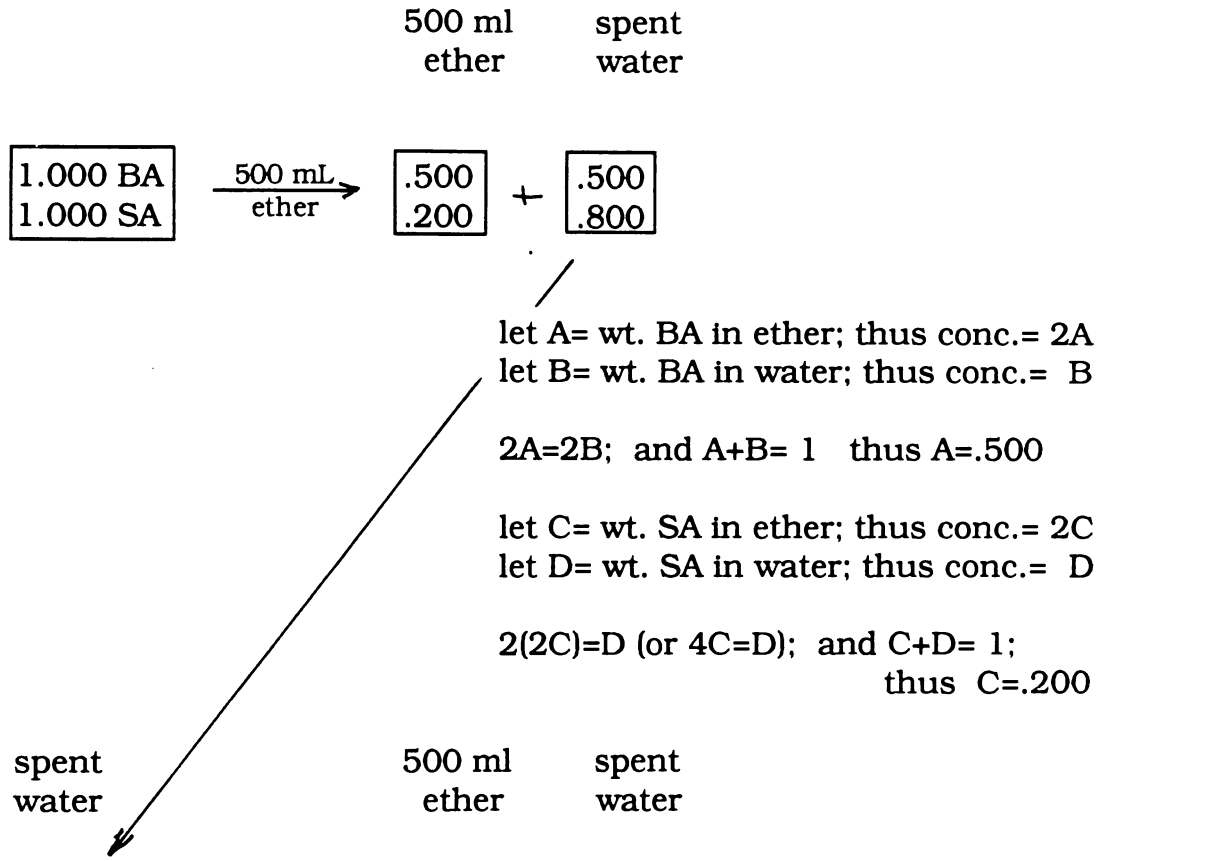
EFFICIENCY AND YIELD OF EXTRACTION: A CALCULATION OF HOW TO BEST UTILIZE AN ORGANIC SOLVENT

Assume: Benzoic acid (BA) has a partition coefficient (ether/water) of 2.0, and salicylic acid has a P.C. (ether/water) of 0.5. With a water solution of 1 gram of each acid initially in the liter of water, how can one best utilize 1 liter of ether?

With a single 1 liter extraction:

1 L. water		1 L. ether		spent water	(in ether) YIELD	PURITY
1.000 BA 1.000 SA	1 L. ether →	.667 .333	+	.333 .667	66.7%	66.7%

With two separate 500 ml extractions:



	(in ether) YIELD	PURITY
.750 BA .360 SA (or 1.110 g of stuff in ether)	75.0%	67.6%
With three 333 ml extracts:	78.8%	68.05%
With four 250 ml extracts:	80.2%	67.99%

It is apparent that with a fixed quantity of extracting solvent, there is an optimum strategy for the removal of a product with the highest purity.

CHROMATOGRAPHY:

The last lecture was directed to the question of the separation of drugs from tissues, by methods of extraction, of partitioning, of selective absorption and desorption. This lecture is directed to the companion part of that project, to see what has been done.

There must be a feed-back from any analytical scheme, allowing the analyst some measure of the effectiveness of his efforts. The tools used here can be divided into the non- destructive (those which allow further analysis on the proffered sample) and the destructive (those which consume the sample in some destructive way). An alternate division is one which extends the separation technique (chromatography) and subsequently reveals compositional character (spectroscopy). Both techniques contain examples of both non-destructive and destructive analyses. In this lecture, I would like to discuss chromatography.

Chromatography is the perfect demonstration of the application of the partition coefficient to analysis. Every example contains within it a demonstration of this concept. In every case there is something that is moving, and the sample to be assayed (or the mixture to be resolved) wants to move with the moving phase. And in every case there is something that does not move, and the sample to be assayed wants to rest with the non- moving phase. A characteristic ambivalence, a wanting to both move and not move, produces a behavior that is a compromise. In separative extraction one chooses the solvent pair (or the conditions) that separates the wanted drug from the junk – in chromatography one chooses the stationary and the moving phase to separate the wanted drug from the junk. Everything is still simply partitioning, but a much greater degree of resolution is obtainable.

Liquid-liquid Counter-current Partition Chromatography:

This is a now totally obsolete technique that was built as an automatic extraction procedure. The equipment, for its time, is probably the most impressive unit of glass blowing ever put together. It is an array of up to 500 delicate units, mounted side by side, that are constructed so that after shaking (the entire 500 units as a unit is shaken simultaneously) and standing until the phases separate, the unit can be inverted allowing the top phase to go in one direction, the lower phase in the other. Then the unit is shaken again. After a few hundred cycles, the phases can be separated in sequential tubes, and there will be a distribution of the components of a mixture that can reflect truly small differences in partition coefficient. The modern-day techniques of preparative chromatography have left this procedure as history, but many chemistry or biochemistry departments still have their "counter-current machine" locked away in a back room somewhere. No one could bear to discard this piece of art.

Liquid Chromatography (LC):

The first demonstration of the technique of chromatography was made in the early 1920's where it was observed that if a solution of plant products (I believe it was the green chlorophylls of geranium leaves) were absorbed on the top of a finely ground solid matrix (alumina) that was wet with that solvent, and then fresh solvent allowed to pass through the column by gravity, that the green layer would separate into several green layers as it passed down the column. As this was green, and visible, the term chromatography was given it (chromos- color; graph- to write).

Many solid matrices have been used – alumina, silica gel, charcoal florasil, celite, starch, talc, cellulose, ion-exchange resins, non-ionic resins – and the moving (solvent) phase is usually organic, although considerable water can be employed if it is compatible with the solid phase. There is much art in the packing and caring for liquid chromatography columns (care in evenness of packing, uniformity of particle size, exclusion of air bubbles, never go dry, sample application, shrinking or expansion of the solid matrix) but with the art in hand, extraordinarily high resolution separations have been achieved.

Much of the practical laboratory separations in toxicology employing column chromatography is largely either "all or none" or "get the fast mover."

The first procedure (all or none) is used to isolate a fraction and clear it of certain classes of contaminants. For example a urine sample, if passed through a cation-exchange resin in the hydrogen form, will have all basic components ion- exchanged onto the resin, whereas the neutral and acidic components (and inorganic anions) will pass on through or can be washed through with water. Then, displacement with 4N or 6N HCl will displace these basic components. Another example can be seen with the XAD non-ionic resin. Its property is to absorb neutral organics. So if the urine is adjusted to a pH of about 8, most basic drugs present will be substantially unionized, and can be absorbed on the surface of the polystyrene resin. Then, water washing will clear out the inorganic salts, and probably some of the strong organic acids. Then, elution with an organic solvent (say, methanol containing ammonia) will remove the absorbed bases.

The second procedure (get the fast mover) actually employs a bit of the chromatographic capability of the column. Usually employing TLC (to be covered in detail in lecture #13), a substrate and solvent system can be found that lets the drug of interest be the fastest moving component. Then, the use of a short packed column of the same substrate, and using the same solvent system, a preparative isolation can be made without the usual care needed for quality chromatography. The faster moving component on TLC will of course be the first thing off the column, and later things are usually overlapped and not appreciably purified by this procedure.

There are small, disposable units now available for just these sorts of manipulations. They contain a couple of grams of solid matrix and are shipped dry.

The extremely high quality resolution work mentioned above is usually achieved with mixtures that contain components that vary slightly in a fundamental physical property. If that property can be emulated in a gradient change in the solvent, then fine and extensive analytic separations can be made. An example of this is the separation of the alpha-amino acids on ion-exchange resins with the use of a buffered elution solvent that has a gradient of a gradually changing pH emulated in some gradient change.

Paper Chromatography (PC):

An entirely different concept of chromatography was developed in the mid 1940's used primarily in conjunction with the separation of amino acids in the area of biochemistry. The initial studies used a thin strip of porous but uniform paper (at the time Whatman #1 was very popular) in a thin strip that was held inside of a small vertical air-tight tube with the sample to be resolved applied near the bottom of the paper, and a supply of an organic solvent in the bottom of the tube. Once vapor saturation had been achieved, the paper was lowered into the solvent, and there was a capillary ascent up the paper. The solvent, of course, was the moving phase, and the paper was the stationary phase. The principle of partition again effected a separation of components up the paper according to the relative coefficients of the components present.

Two different types of modifications appeared soon thereafter, greatly expanding the versatility of the procedure. Two dimensional descending paper chromatography, and preparative chromatography.

Using an overhead trough in a closed tank, it was found that the paper, here a sheet of perhaps two feet square, could be suspended and with the sample to be resolved placed at one corner and solvent placed in the trough, a chromatographic separation could distribute the components of the mixture down one side of the paper. Then, after removal from the tank, and thorough drying, the paper could be reintroduced at 90 degrees to the original position, and with a second solvent employed, this one dimensional line of components redistributed in a second dimension, effectively covering the entire surface of the paper. This display could be developed with an appropriate indicator at this point (usually ninhydrin with amino acid mixtures), yielding a pattern of spots which were easily identified and really reproduced. Usually a basic solvent was used in one direction (a collidine or lutidine) and an acidic solvent in the other (perhaps butanol and aqueous acetic acid). At about this time the tracer element carbon-14 was just coming into research, and a commonly employed technique was the two dimensional chromatographic display of radio-labeled amino acids (from a protein hydrolysate, for example) coupled with an exposure of the paper to X-ray film giving a photographic display of the distribution of the beta-emitting carbon on a transparency that could be superimposed. This was a time of remarkable forward progress in biochemistry.

The preparative technique employed a descending paper chromatographic technique again from an overhead trough, but here the bottom of the paper was serrated allowing

the solvent to drip into individual vials, one per serration. Then a potential difference was applied across the paper horizontally allowing an electrophoretic migration to be effected laterally. Thus as the solvent chromatographed things vertically, the electric migration chromatographed things horizontally, and the process could be continued (in theory) continuously so that with a continuous application of material at the origin, preparative isolation of samples differing in pH character and in polarity could be separated. This process never was very practical.

Thin-Layer Chromatography (TLC):

This technique is again a fine example of partition being put to work. Here the moving phase is the solvent combination that ascends a fine mesh of solid material that is firmly attached to a solid surface. The ascending phase is the moving phase, an organic material or mixture, chosen for a polarity that will effectively compete with the solid phase material, the stationary phase. The solid surface is currently usually glass, but flexible plastics have been used.

The techniques of TLC have become the major inexpensive tool for rapid analysis with many chemical laboratories and analytical groups. A more complete discussion of choice of substrates, selection of solvent combinations, developing reagents to visualize the separations achieved, two dimensional procedures, *and* practical problems encountered with its use in drug screening, will be covered in Lecture #13.

Gas-Liquid Chromatography (GC):

The major quantitative chromatographic technique used today is gas chromatography. This technique was developed in the 1950's by two natural product chemists right here in the Bay Area, for the resolution and characterization of the flavor components of strawberries. With this technique, the partition procedure involves the use of a gas as a moving phase, and of a liquid film on a solid substrate as the stationary phase. In the early days, it was assumed that the entire system was not much more than a small-scale distillation, and that things were separated according to their boiling points. Hence, the early name, VPC (or vapor-phase chromatography). And there are analytical schemes that do call upon just these physical properties. Later, we will discuss the use of GC in the determination of blood alcohol. The separation of ethanol from methanol and propanol does depend completely upon these relative boiling points.

But it is now recognized that there is a true absorption- desorption process in operation here, and that partition plays a major role in its explanation. And since the moving phase is a true vapor phase, the mixture being separated must be with a real vapor pressure.

The earliest systems employed a packed column. This was a long helical tube of glass or metal (five or ten feet, perhaps) that had been packed with finely ground solids (inorganic, inert, and reasonably uniform in size) which had been coated with

a high-boiling liquid. The earliest system I had put together employed a ground-up flower pot covered with stop-cock grease. It worked.

While an inert gas is flowing through the column, a solution of a mixture of compounds is applied to the front of the column, and there is a percolation down the column that separates the mixture into its several components. The component least absorbed on the stationary phase (the partition coefficient greatest towards the moving phase) emerged first. The others followed again according to their conflict between the oil and the vapor. This is called packed-column gas chromatography, and it is still widely used. A major problem remains undiscussed – how does one see the order of emergence from the column? Here a detector is used, and this was the first area of explosive development in GC analysis.

A number of different detectors have been developed (thermal conductivity, flame ionization, electron capture, nitrogen- phosphorus, mass-selective detectors) and they will be discussed in detail in Lecture #14, on GC. There, too, will be presented the modern variants of GC such as capillary columns, temperature programming and sample derivatization.

High-pressure Liquid Chromatography (HPLC):

An analytical procedure that is a companion to GC for the quantitative analysis of mixtures is HPLC. This is again a liquid chromatographic technique, employing a solid phase of fine mesh particles and a moving phase of an organic solvent mixture. But here, as opposed to the early classic LC, the ration of length to diameter is considerably larger, the particle size is considerably smaller, and due to the consequent back pressure there is a high head pressure needed to achieve a usable flow rate.

The advantages of this technique are the ability to separate mixtures of materials that are without appreciable vapor pressure, and to use solid substrates that can range from one extreme to the other in polarity. Also, when water can be tolerated in the solvent system, the ability to analyze an aqueous sample such as urine directly without preliminary extraction or cleanup, makes the operation attractive for inexperienced analysts. The major disadvantage is the costs and care that must be taken to allow solvents to be pumped through extremely fine tubing at often extremely high pressures. The practical aspects of HPLC analysis will be discussed in Lecture #14.

There are many subdivisions of chromatographic techniques encountered (flash chromatography, low-bar preparatory chromatography, centrifugal chromatography, prep-GC,) but these are simply modifications of the above techniques, and will be discussed along with them later.

SPECTROSCOPY

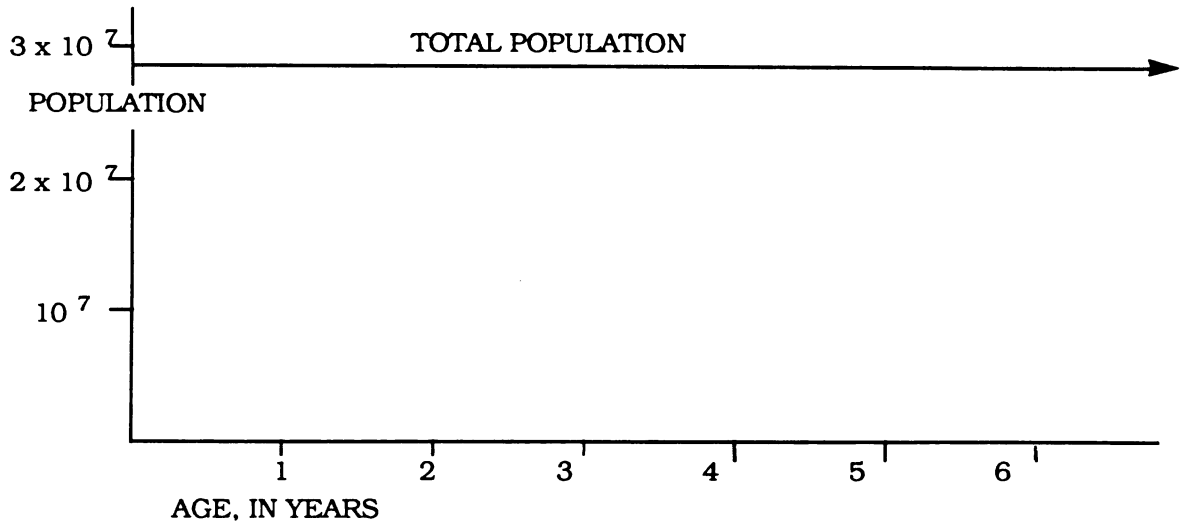
In Latin, the verb "specere" means "to look at", and from this came the noun "spectrum" meaning "appearance." In current popular use, a spectrum is an array or spread of things usually presented in a continuous pattern. In the scientific area, the spread is composed of a continuum of a form of energy, either to be emitted or to be absorbed.

"-Scopy" comes from Greek and means viewing or observing. Thus spectroscopy means to view a spectrum and a spectroscope is the instrument for this viewing. Similarly, "-graphy" means writing, leading to spectrography meaning to record a spectrum with a spectrograph, and "-metry" means measuring, leading to spectrometry meaning to measure a spectrum with a spectrometer.

Every form of spectroscopy is, in its ultimate analysis, a matter of counting events. Regardless of the energy field being assayed, one can define it only by looking at a small portion of it for a period long enough to tally events that are revealed there, then moving to an adjacent portion for additional tallying, and on and on until the desired field has been analyzed. Later, I will discuss several forms of spectroscopy specifically, but first let me try to paint a word-picture using as an example something far from instrumental analysis. My intent here is to present the concepts of noise (signal to noise ratio) and resolution.

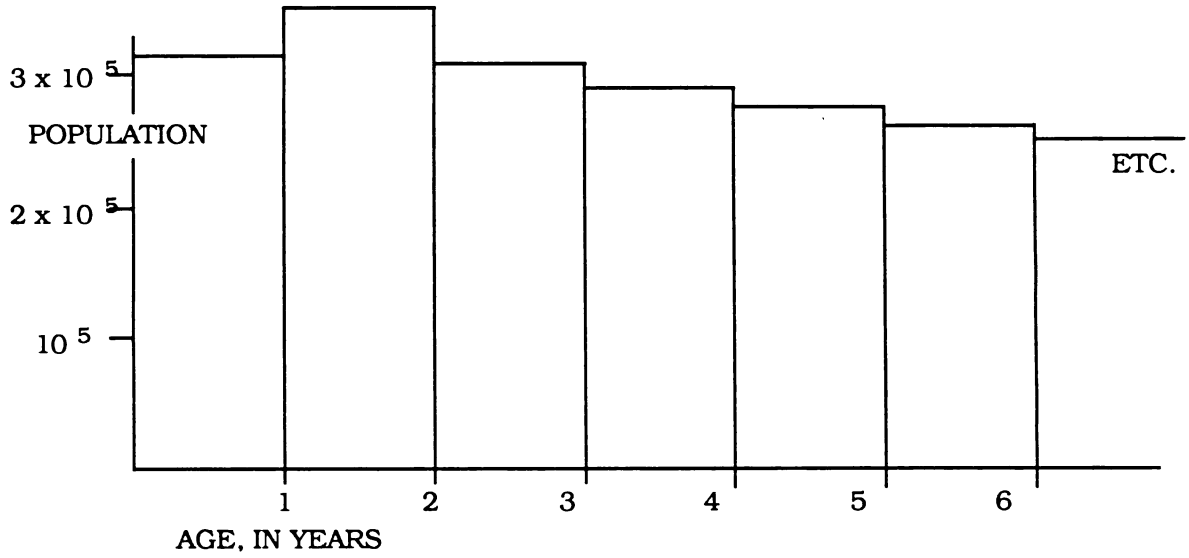
Let a horizontal line (the abscissa or X-axis) represent the continuum to be analyzed, and in this example let us say it portrays the population of California. Let the Y-axis (the ordinate) be the tally of events. The goal is to determine the spectrum of age distribution of the people who live in California.

If one were to look at the entire field (all the people) at the same time, one would



nicely measure the sum total of events involved, but there would be no knowledge of how they were distributed. The tally here would be the total population.

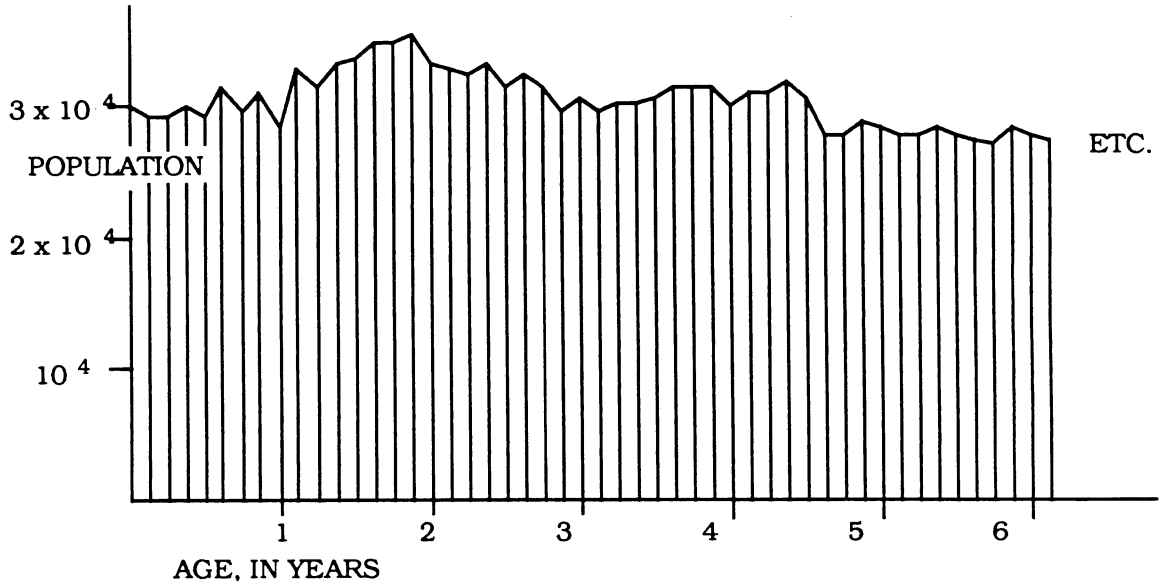
As a more exacting approach, let us divide the X-axis into 100 equal subdivisions which will represent, in this example, people of the age 0 to 1 years, 1 to 2 years, and on and on, assuming there is no one over the age of 100. Then, we will screen the population past the first of these subdivision "openings" watching for babies between 0 and 1 years of age. A tally is made of these events, but they may arise from either of two sources. There are those that are actually viewed through this opening, and those that make their way into the event-counter from everywhere else. Thus, a certain period of time must be devoted to noting how many events are tallied as 0 to 1 year olds, that are actually errors (wrong ages given, liars, people from



Oregon, age-unknowns, etc.) This is known as determining the noise level, and it is here where statistics enters the art of spectroscopy. The total number of events entered on the Y-axis must be the difference between total events and the events that are noise. Total events minus noise events is the net valid tally, and total events divided by noise events is the signal-to- noise ratio. Clearly, the closer this latter ratio is to unity, the larger the number of total events collected must be, to be statistically believable. So how long do you look at this sometimes utilized and sometimes ignored "opening?" This obviously depends on the number of valid events needed, and the smaller the number of total events occurring, and the smaller the signal-to-noise ratio, the longer it will take to get a believable number. And once that number is obtained, then go to the second opening (the 1 to 2 year olds) and repeat the process. When all tallies have been completed, a plot of each side by side (a histogram) represents the spectrum. It will be of only fair resolution since it represents the connecting of tallies each representing a full 1% of the territory to be viewed.

A much finer (higher resolution) spectrum can be obtained by dividing the X-

axis into 1,000 subdivisions (i.e., making each opening a tenth of a year or about a month which is the same as saying we will use a more narrow slit. Now, as an example, a tally must be made for all people who are between 0 and a month old, or between 22 years 4 months and 22 years 5 months of age. It will be necessary to take much more time to assimilate a statistically valid number of events (as described above) but the resulting population spectrum will have a greatly increased resolution.



Thus, in practical terms, the use of a spectroscope requires making compromises. The narrower the slit, the higher the resolution will be but the longer everything takes. And if the picture being sought changes with time, this must also enter one's consideration of instrumental parameters.

ELECTROMAGNETIC RADIATION SPECTROSCOPY.

One of the most important regions to be considered in the scientific area is the electromagnetic radiation spectrum. This is an extraordinary continuum extending without limit in both directions. Towards the short-wavelength, high frequency direction of the spectrum there are the cosmic rays and gamma rays, with intense energies. As one passes along the spectrum in the lower energy direction one encounters X-rays, then ultra- violet, visible, infrared, and microwaves. Yet further are the radiowaves more familiar to TV-watchers and finally to AM radio- listeners. But the stretch between the cosmos and KSFO embraces many orders of magnitude of frequency change, and no practical instrument could be designed to cover everything. Rather, small and specific instruments are used to survey portions of this broad spectrum for particular needs. Several of these are briefly described here.

Gamma-ray Spectroscopy:

The X-axis is the energy contained in a gamma-ray, and the Y-axis is the

number of gamma-rays observed falling into any defined energy range. What one obtains is a spectrum of the distribution of gamma particles (from a decaying radioactive sample, for example) based upon their energies, and from this there is an identification of the radioactive species present. Or, if a mixture is present (mother-daughters for example) an insight into this relationship can be made from the change seen in the spectrum as a function of time.

Ultra-violet Spectroscopy:

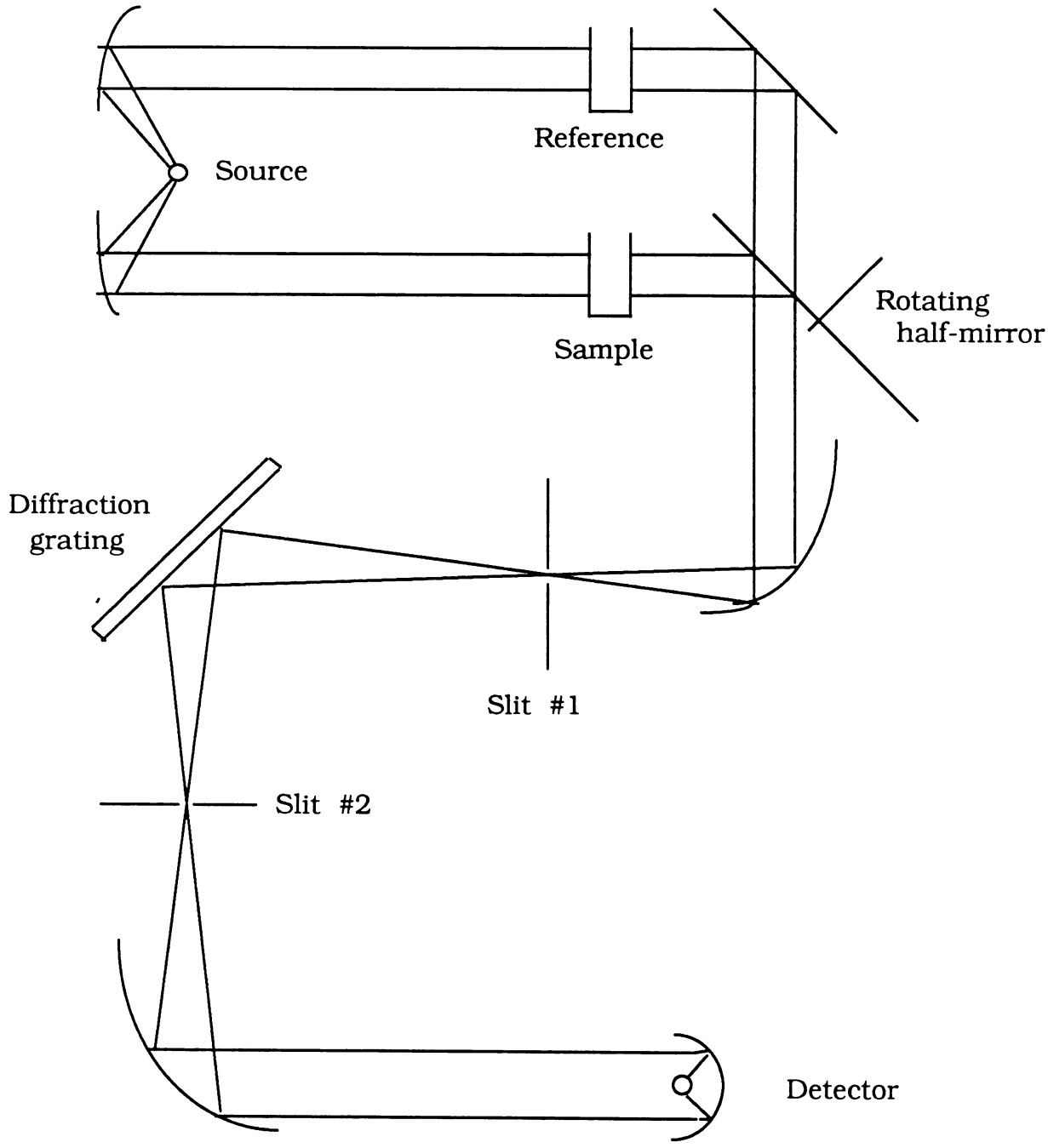
One of the most common spectrographs is the single unit that presents the ultra-violet and the visible spectra as a continuous display, with wave-length on the abscissa, and optical density on the Y-axis. The discussion between optical density and percent transmission will be discussed below, but here it is important to appreciate that whereas the horizontal axis is some measure of wave-length (millimicrons, Angstrom units, reciprocal of frequency), the Y-axis is a measure of the number of photons that are allowed through the slit (not absorbed by the sample under consideration) which can eventually reach the detector. Two major dimensional considerations must be considered.

The X-axis represents, quite literally, the energy of the photon being absorbed. Photons that are absorbed in the ultra- violet (the region more energetic than the visible) are related to electrons that are involved in the bonding structures. At very high frequencies (the low 200's) one sees energies associated with the bonding electrons. Then, at less severe frequencies there are the bonds of olefins, and eventually the conjugated polyene systems of the dyes and extended aromatics that are colored since they absorb in the visible portion of the spectrum. An entire art has been developed around the deduction of structure by the exacting nature of the ultra-violet absorption.

And having established identity, the Y-axis deflection can serve as a quantitative measure of the amount of the absorbing species that is present. This can be done through percent transmission or optical density.

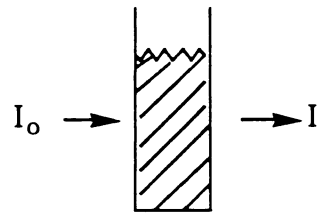
The "null" recorder is a spectrograph that determines the effective percent transmission of a test sample. In such a machine (most common with infra-red spectrophotometers) a uniform comb is inserted into the reference side of a double beam spectrophotometer until the two beams (the reference and the sample beam) are of equal intensity. The position of this reference-beam comb then dictates the position of the pen on the spectrograph. The percentage transmission is recorded, the system is non-linear, the system is relatively dead, and the system is very cheap. A typical machine lay-out of an infrared spectrophotometer is shown on the next page, and will be explained in the lecture.

The "ratio" recorder is a spectrograph that determines the effective opaqueness of a test sample. In such a machine (most common with ultraviolet spectrophotometers) the difference between the two beams (reference and sample) is taken as a signal, amplified to a usable level, and employed as a voltage to drive the pen on the recorder.


Reference
Source
Sample
Rotating
half-mirror
Diffraction
grating
Slit #1
Slit #2
Detector

The optical density is recorded, the system is linear, the system is completely alive, and the system is relatively expensive.

The relationship between percentage transmission and optical density is logarithmic. A completely transparent sample has a transmission of 100%, and has an optical density of 0. If the transmission is reduced by a factor of 10, the density is increased by an order of magnitude, and is now 1. With a decrease of a factor of ten again, the %T is 1%, and the O.D. is 2. A completely opaque sample is 0% transmission, and has an optical density of infinity. These useful equations are good to have at hand, for the conversion of optical density to %T or the reverse conversion. In both cases the optical density is defined as the loss of input energy due to absorption by the sample. The absorption constant is also defined. A couple of other related equations are also listed below.



$$OD = \log \frac{I_o}{I}$$

$$\text{or } \%T = \frac{I}{I_o} \times 100$$

$$E = h\nu$$

$$\nu\lambda = c$$

$$I = I_o e^{-al}$$

E = energy/quantum
h = Planck's constant
ν = frequency
λ = wave length
c = speed of light
a = absorption coef.
l = length of cell

Fluorescence Spectroscopy:

A measure of the fluorescence of a sample is in many ways a reflection, a mirror image of the ultra-violet absorption. Rather than being exposed to an ultraviolet photons in the U.V. spectrophotometer, a sample is excited with an intense ultraviolet source, and the release of this absorbed energy is in the form of photons of lower energy (longer wavelength) which can provide an emission spectrum. A compound must absorb to emit, and often this emission is of a greatly enhanced intensity. Thus there is a broad application of this technique in the area of polycyclic hydrocarbons and other fluorescent species.

Infra-red Spectroscopy:

Absorption in the infra-red portion of the radiomagnetic spectrum is a measure of the interactive action of particles the next larger than the electrons. Here one deals with atoms, and -the lightest of the possible atoms (hydrogens) in vibrating interaction within an organic molecule must be with a heavier atom, namely a nitrogen, a carbon,

or an oxygen. All of this starts at about 2.8 microns (2800 millimicrons) so the spectrum of electromagnetic absorption is totally clean between the last of the visible absorbers (maybe 600 or 800 or so millimicrons) and 2800. This is not exactly so, as the earliest instruments could measure something at the 1400 millimicron area, then called the near-infra-red, which proved to be weakly displayed overtones of the primary infrared absorptions.

The absorption between the first of the H-something stretches and the last of the heavy atom - heavy atom stretches (about 6 or 8 microns (6000 - 8000 millimicrons, or 1200 to 1600 wavenumbers, or reciprocal centimeters, cm^{-1}) constitutes the functional group area of the infra-red absorption. From there on out to the end of the usual spectrum, one has the "fingerprint" portion that reflects the identity of the molecule as a unit. This latter has no easily assigned functionality. A comparison of absorption within this latter portion of the spectrum constitutes one of the most convincing presentations of chemical identity.

Raman Spectroscopy:

A presentation of spectral information very similar to the infra-red is presented in the Raman system, where there is the excitement of molecular emission (with an intense U.V. pulse) rather than the absorption of infra-red transmission (from a broad-spectrum glower). The information obtained is substantially similar, and the infrared is cheaper and more reliable.

Optical Rotary Dispersion:

Here, as with ultra-violet spectroscopy, the X-axis is wavelength or frequency but the ordinate is a measure of optical rotation. When the U.V. absorption spectrum is measured with the exposure to optically polarized light, the nature of the absorption curve can give insight into the absolute optical configuration of the dissolved compound.

Nuclear Magnetic Resonance:

The X-axis here is a magnetic field which is being swept with a radiomagnetic signal. When there is absorption of energy from a proton that is spinning in resonance with this energy source, a Y-axis deflection records this absorption. A measure of the molecular environment of this proton can be deduced from the location of this absorption. Similarly, the absorption of energy from a free electron in a radical compound in solution can describe the environment or the tumbling nature of the radical molecule. This latter is called electron paramagnetic resonance, or EPR spectroscopy.

Mass Spectroscopy:

This spectroscopic technique will be the subject of a later lecture (#16) and will not be discussed here.

THIS IS A COPY OF THE FIRST OF THE TWO MIDTERMS, GIVEN IN 1985

Forensic Toxicology
BEHS 183 Lect. #10
Midterm #1 9/26/85
Open Book

Answer briefly (in two sentences or so):

(1) Compare the effects of a fixed positive charge in a drug (as in a quaternary ammonium salt) and a pH-dependent positive charge in a drug (as in a tertiary amine salt) on its partition between organic and aqueous solvents.

(2) What are the fatal flaws in the approach to a problem (in the forensic area as opposed to the mathematical area) that depends upon the search for positive proof?

(3) Employing ion-exchange resins, give a brief procedure for the conversion of a solution of ammonium bromide into an equivalent solution of ammonium chloride.

(4) Describe the relationship between the compound isoprene and the chemical class of terpenes.

(5) Many animals studies are required before there is an eventual approval of a drug for prescription availability by the FDA. Define or describe the following which may be associated with this process:

(a) Chronic toxicity

(b) ED-50

(c) Therapeutic index

(d) Plasma half-life

(e) FDA

(6) What are the positives and what are the negatives that are presented in a discussion of the value of obtaining extensive LD-50's in animals?

One of the most severe drug problems that we must live with today is the widespread use of alcohol. Although the social and medical aspects of alcohol use are severe, the major concern of the legal community is its association with driving. Every state has its own definition of DWI (driving while intoxicated) and this consideration has been complicated by the legal and social awareness of the impairment of performance that can result from drug use. The DUI (driving under the influence) is a legal reality now in California.

THE LEGAL DEFINITION OF ALCOHOL INTOXICATION

This is a number that is explicitly given in the current law. If a person has a blood level of 0.08 g% or higher, he is intoxicated and may not legally drive. Let me define a few things.

"0.08"

Some comment on this number is needed. By law, only two digits can be used to the right of the decimal point, and in any calculation that generates a third digit, this must be dropped. Thus, a finding of 0.129 g% blood alcohol can only be reported as 0.12 g%.

"g%"

This is a good time to introduce the several dimensions that are used in the measurement of body levels of drugs. The term g% means, literally, grams per centum, or grams per 100 grams, or simply percent. Although fluids (blood, urine) are usually measured volumetrically, the specific gravity is close enough to 1 to allow an interchangeability of dimension here.

Occasionally one sees some variations of this term. mg% used to be found, usually in clinical medicine, and represents milligrams drug in 100 ml of blood or urine. And with a thousandth of this, one finds ug% or micrograms per 100 ml. In the older literature one encounters a similar form. This is g o/oo rather than g o/o and it represents grams per millum, or grams per thousand grams (or 1000 ml).

This latter term is today usually given as ppt, or parts per thousand. This abbreviation is again modified in magnitude jumps, to ppm (parts per million), and ppb (parts per billion). This representation is currently used largely in the descriptions of trace contaminants, poisons in the environment, residues on crops, etc.

In toxicology, today, the standard terminology is the expression of weight per ml. In the following table, each line is an order of magnitude (an order of magnitude is synonymous with a factor of ten).

neat			1
			0.1
g %			0.01
g o/oo	ppt	mg/ml	0.001
			0.000,1
mg %			0.000,01
	ppm	µg/ml	0.000,001
			0.000,000,1
µg %			0.000,000,01
	ppb	ng/ml	0.000,000,001
			0.000,000,000,1
			0.000,000,000,01
	ppt	pg/ml	0.000,000,000,001

The laws on blood alcohol are written in terms of g%, and that is the phraseology used here.

"Blood"

All laws related to drunken driving define intoxication levels as concentrations to be found in blood. This is the primary definition, but as analyses can and are made in other body fluids, legally defined conversion factors also provided to reduce their observed concentrations to blood equivalents. Hence, a found urine level will be divided by the factor 1.3 before being reported as a blood level, and a found breath level will be multiplied by a factor of 2100 before being reported as a blood level. These are the conversion factors in California law at the present, where the stated level of intoxication making driving illegal is 0.08 g%, in blood.

The relationships between alcohol intake, body level, and time are complex and often highly individual. Nonetheless, these considerations are often important in the legal processes associated with prosecution.

A frequently asked question is, how many drinks does it take to get you drunk? A good idea of the dimensions involved may be had by visualizing the subject and the drink in rounded-out terms. If the subject weighed, say, 80 Kgs, and if he is half water, then he could be viewed as a bag containing 40 Kgs (something over a cubic foot) of water.

The quantity of alcohol to be found in alcoholic drinks is quite calculable. Hard booze should be 86% proof. (In pre-analysis days, a common convention, used to insure that your scotch had not been diluted dishonestly, was to wet gunpowder with it and determine if it would still ignite. Eighty-six proof — exactly 43% — was the break point). Current hard liquor is frequently 40% in strength. Wines can range from 12 to 20%, and beers and ales from 3 to 8%. But in general, one cocktail (with one and a half ounces of scotch or vodka), one glass of wine (with 4 ounces of pinot noir) or a bottle of beer (one 12 ounce service of Henry Weinhard), any of these, will provide about 16 grams of ethanol. And about two and a half of such drinks will provide about

40 g of ethanol which, if dumped into the plastic bag containing 40 Kg water, will provide a concentration of 0.10 g%. This level of drink intake already exceeds the maximum alcohol level that allows one to drive legally.

The alcohol can be very rapidly absorbed, some from the mouth and nose via inhalation, and quite a bit from the stomach. Blood levels are established relatively quickly depending on the quantity and rate of ingestion. Small amounts may show a blood maximum in a half hour; the consumption of 50 g as a bolus may require 2 hours to achieve a blood maximum. The loss of alcohol from the body is widely assumed to be linear, i.e., independent of the amount consumed. Some 95% of the ingested ethanol is converted to carbon dioxide, and this metabolism occurs in the liver proceeding at a pace of between 5 and 10 g/hr. Thus following a 40 g input it will take between 4 and 8 hours to be alcohol-free. In terms of blood levels, this reflects a concentration drop of perhaps 0.015 g%/hr. However, careful studies have weakened this assumption in that there appears to be some dose dependency at low blood levels. This factor will rarely be important in legal cases however. It must be remembered that the alcohol that is seen (in blood, or urine, or breath) is only a very small amount of the ingested ethanol that is ever excreted (between 0.1 and 0.5%).

Ideally, a person's social risk as a driver should be determined by demonstration rather than by blood level of a chemical. There is no question but that some drivers are competent with blood levels at or above 0.08 g%, and that others are incompetent even when they are alcohol-free. Some efforts are directed towards this end by the administering of a field sobriety test which calls upon demonstration of motor coordination and cognitive integrity. But these measures are largely confirmatory and used as supplementary evidence to reinforce the blood alcohol value found. Further, when there are indications of impairment with these coordination tests without the usual signs of alcohol (breath smell and flushed facies) there is reason to assay a urine specimen for other drugs.

California law now puts a blood level of 0.08 g% as evidence of a driver being "presumed drunk." Prior to 1982, the suspected drunk driver had the choice of analysis, either blood, urine, or breathalyzer, but since then, a choice is allowed only if more than one test is available.

There are pros and cons with each of the test fluids allowed. Blood is primary in legal importance, and of course does not require any conversion with an arbitrary correcting factor for court presentation. However, it is necessary to have a licensed technologist perform venupuncture, and there is a considerable expense having this service available around the clock.

Urine analysis is less invasive and can be collected with a less medically involved procedure. However, the immediate content of the bladder is not a fair measure of blood alcohol, as the collected urine has accumulated over an unspecified period of time. It may be unduly high since it had accumulated from an earlier, more intoxicated period, or it may be unduly low as some urine may have cleared prior to any alcohol consumption. Thus the law requires that the bladder be voided, and after

a waiting period of at least twenty minutes, a test sample of urine be taken. A few ml can almost always be generated, and failure to produce this constitutes a failure to cooperate, and can lead to additional charges. Further, a small but occasionally raised point of uncertainty is the validity of the required conversion factor, 1.3 to 1; urine to blood. This, of course, will vary from person to person, and possibly for a single individual from day to day.

Breath analysis is becoming the procedure of choice, in that there is no invasion and little medical objection that can can be used on the scene of a potential arrest. The result can be obtained on the site, and so subsequent police action can be decided upon from findings at the moment. However, serious problems are possible. Besides the needed conversion factors, there is the need of achieving true alveolar air, that which is in the most intimate association with the blood. All of the initially expired air will be diluted with the most recently inhaled air. Further, any oral ethanol (from recent drinking, vomiting, or mechanical retention) will exaggerate the findings. And if the procedure involves spectral measurement, problems of moisture content and temperature are real. A final problem. Unlike the blood and urine sampling, there is still no satisfactory procedure available for obtaining and maintaining a duplicate sample of breath for subsequent confirmation. The results of the first findings cannot be challenged later by an independent analyst.

GAS-CHROMATOGRAPHIC ANALYSES

The best established and most widely accepted analytical procedure is gas chromatography. Three general procedures have evolved, each with its own virtues.

The most direct and least complicated procedure is the analysis of either blood or urine by the direct injection of a sample into the chromatograph. Characteristically, a column is used that is responsive simply to boiling point, and advantage is taken of the fact that, other than water, there are rarely any volatiles found in either fluid. Occasionally methanol is encountered (from sterno or bad liquor) or isopropanol (from intentional ingestion of rubbing alcohol) or acetone (from metabolic sources). But these are rare, and easily distinguished from ethanol. Commonly employed internal standard are propanol or methyl ethyl ketone, as they are easily separated from ethanol and are very rarely encountered as a competing poison. One adds a known amount of propanol as the internal standard to a blood sample that contains a known amount of ethanol and, separately, to the blood sample to be analyzed. Then, in separate injections in the GC, separate ratios of ethanol to propanol may be determined. Since the amount of propanol was constant, it will correct for dissimilar quantities of injected material, since ratios only need be considered. And as the amount of ethanol in the standard was known, the amount in the unknown sample can be determined.

A similar process has been used but with the employment of an extracting solvent. This avoids the complications that can follow the continuing deposition of debris from urine (salts) or blood (charred protein). n-Butanol, toluene, and n-propyl acetate are examples of extracting solvents. As ethanol is more volatile than any of

these solvents, it emerges from the GC column first. Intrinsic disadvantages of any extraction procedure are the need of allowing the extraction solvent time to completely clear from the column, and the uncertainty that can come from temperature and concentration influences on the extraction coefficient (the partition of alcohol between the aqueous and the extracting solvent).

Head-space analysis is currently very popular, as it is easily adaptable to automation. Acetone, methyl ethyl ketone, and dioxane have been employed as internal standards. Following the addition of a known amount of internal standard to an unknown blood or urine, the sample is placed in a sealed tube and warmed to a given temperature for a given period of time. Then a sample of the air above the sample is injected into the GC, and the relationship between the ethanol and the internal standard allows the determination of the sample level. This procedure allows the cleanest possible operation, and commercial instruments are available with rotating turrets and constant temperature baths, allowing the automatic processing of dozens of samples sequentially. A major disadvantage is the extreme dependence on temperature and time reproducibility.

The techniques of head-space analysis are suitable for the analysis of breath samples as well. A number of instruments have been developed intended for analysis on site of a suspect's breath. As mentioned earlier, there is as yet no satisfactory procedure for retaining representative breath samples for later analysis.

CHEMICAL ANALYSES:

Almost all chemical analyses of blood or urine samples depend on the removal of alcohol from the aqueous sample (distillation) followed by its quantitative oxidation. This latter step is usually achieved with dichromate as this allows a visible qualitative measure by exploiting the color change from yellow-orange (chromic ion in acidic dichromate) to blue-green (chromous ion). There is a long history of the development of the oxidative determination, but the name most often associated with the process of distillation and oxidation is Widmark. His process involved the heating of a sealed tube that contained, in separated sections, both the sample to be analyzed and a dichromate solution. The alcohol would distill into the dichromate, and the excess oxidant determined by sodium thiosulfate titration to a starch-iodide endpoint. Many variations on this theme have been used, both to increase the reproducibility of distillation, and to minimize interference with other volatiles which are distillable, such as acetone. Yet other potential interfering agents (such as ether, chloroform and paraldehyde) react very slowly with dichromate, and are not found at levels as high as those found with alcohol.

Rather than employing titration, several methods are in use that exploit the color changes associated with oxidation with spectroscopic techniques.

These procedures are eminently suited for breath analysis, as the alcohol is already in the vapor phase and the process of distillation is not needed. The marketplace is filled with instruments with names with almost every possible

combination of the prefixes drunko-, intoxi-, breatha-, alco- , sobri- and the suffixes -lyser, -analyser, -meter. Again, a colorimetric procedure is usually used, and although the breath sample cannot be retained, the reaction medium can be, and has been submitted as confirmatory evidence in court.

BIOCHEMICAL ANALYSIS:

The only practical biological assay for blood alcohol has employed the enzyme alcohol dehydrogenase, with the end point usually determined by the spectrophotometric measurement of the NADH generated. Methanol and acetone do not interfere, and propanol and butanol only to a very small degree. The high sensitivity of these procedures has little value in legal cases.

PHYSICAL ANALYSIS:

An additional technique for the determination of the alcohol content of breath has employed this use of infra-red absorption. Exploiting the intense C-H stretching absorption at 3.39 microns (2950 cm^{-1}) a measure of the "hydrocarbon" content of the breath can be made, and both the methyl and the methylene group of ethanol absorb strongly in this spectral region. There is the potential of interference from unexpected hydrocarbons that might be present, but this is claimed to be a very rare complication. A more serious problem is the erratic accumulation of breath moisture on the internal surfaces of the instrument, giving falsely high readings that are difficult to correct for. As the absorption of moisture is always accompanied by the absorption of some ethanol, internal heating arrangements are required.

ANALYSES (OTHER THAN ETHANOL) THAT CAN BE PERFORMED WITH BLOOD OR URINE INTACT

Much of the rest of this course will be devoted to the discussion of analytic procedures and techniques that call upon either the isolation and concentration of a drug from body tissue or fluid, or upon the use of some extraordinary property or trick to allow drug analysis.

There are relatively few drugs that can be analyzed without such concentration or trickery. Alcohol was the topic of lecture #11, where it can be assayed by the direct handling of blood or urine. There are a few additional analyses that are valid at these levels – some again directly on body fluids, and others that require the substitution of water for body fluids.

VOLATILES

In the alcohol lecture, mention was made of the occasional interference with isopropanol or methanol. Both chemicals can be encountered in blood or urine samples. Methanol (wood alcohol) is seen as a component of illegally made alcoholic drink, and it is occasionally accidentally substituted for grain alcohol. It is an intoxicant and CNS depressant very much like ethanol, and although less toxic in acute exposure, it is potentially much more damaging with chronic exposure. It can lead to blindness in man through damage to the optic nerve tract. It is usually analyzed by its oxidation to formaldehyde, for which there are several sensitive assays. Isopropyl alcohol is encountered in cases where rubbing alcohol has been consumed, often intentionally as a way of replacing ethanol. It is probably somewhat less toxic than ethanol, but more prone to cause retching and vomiting. Higher levels than needed for simple depression can cause paralysis.

Glue sniffing has been a recent popular fad, and exposure to the solvents in glue (airplane cement) can often be confirmed by their presence as late peaks in the usual GC ethanol assay. In the same vein, there are periodic outbreaks of the consumption of ether or chloroform, either of which can lead to a highly intoxicated state. As with all the other volatiles, the GC analyses are of great value, and at a practical level, an emergency involving overuse of any of these common volatiles can be identified by their tell-tale smell on the breath.

CARBON MONOXIDE

A more subtle poison is the odorless and colorless gas carbon monoxide. It is one of the three major killers in the United States today. The maximum concentration allowed in an 8 hour day is 100 ppm; 400 ppm for an hour. 200 ppm will generate the primary characteristic symptoms – headache and mental dullness – in about 4

hours, and 600 ppm will lead to unconsciousness in about 2 hours. 1000 ppm will produce unconsciousness in an hour, and will be fatal in about 4 hours.

The mechanism of action is simple. It combines with hemoglobin in much the same way as does oxygen, but the attachment is much stronger than that with oxygen. The product is called carboxyhemoglobin, and since the CO attached there is not metabolized, it can be eliminated only through the lungs, being eventually replaced with oxygen. The toxicity is simply due to an inadequate oxygen supply to tissue, but this is amplified in that carboxyhemoglobin (COHb) can actively inhibit the release to tissue of oxygen from normal oxyhemoglobin (O_2Hb).

Some carbon monoxide is produced endogenously (endo-, within; gen- from genesis, the originate) through the metabolism of hemoglobin itself, but the major portion found in the blood comes from exogenous sources (exo-, outside). Incomplete fuel combustion and cigarette smoking are the major sources.

The expression of symptoms of CO poisoning depends more on the level of COHb generated above the day-to-day baseline rather than on the absolute value seen. Thus, a non-smoking, non rushhour commuter may have a blood level of 2 or 3 % COHb (the base metabolic contribution is perhaps 0.5 %) but the commuter who smokes may show as much as 15 %. An increase of blood saturation by 10% above the individual's baseline is largely symptom free. An increase of from 10 to 20% will produce a tightness across the forehead and a slight headache. At higher levels there is a throbbing in the temples, dizziness, nausea, vomiting, and syncope (loss of breathing and circulation due to cerebral anemia or ischemia) (anemia; a-, without; and -emia, blood; and ischemia; ischaemus, to restrict or restrain; and - emia, blood again). But it is not that blood doesn't get to the brain, it is that the blood that gets there has no oxygen. The net result is the same. The brain is starved for oxygen. Coma with intermittent convulsions, and death, are seen in the 70 to 80% blood level However, the cyanosis (from cyan-, blue, the color of the face with inadequately oxygenated blood) that might be expected from the oxygen absence, is obscured by the cherry- red color of carboxyhemoglobin, which is visible through the skin.

An interesting sidelight is that a person can live without any functional hemoglobin whatsoever, if enough oxygen can be forced into solution in the blood. A person with 100% COHb, if placed in a hyperbaric chamber with pure oxygen at about 4 atmospheres (about 20x the normal oxygen availability) will survive, although he may convulse at these levels.

A common clinical analysis of carbon monoxide is the Winkler assay, employing a procedure of blowing out the CO into cuprous chloride where it is readily absorbed. A rapid spectroscopic assay calls upon the spectral difference between COHb and O_2Hb. Whereas both chemicals have identical absorptivities at 610 nm and again (but higher) at 570 nm, the oxygenated form is the more opaque in between these wavelengths. Hence, if a blood sample from a patient is saturated with oxygen and, separately, with carbon monoxide, these can form a 0% and a 100% standard curve for the determination of the level of the patient's original blood. He serves as his own

control. Remember that the values separating normal and pathological are large, so accuracy is not needed. Speed is more important.

HYDROGEN CYANIDE

The gas HCN is another common poison that presents a similar symptomology, but through a different mechanism. The ion acts to block oxidative mechanisms by reacting with the trivalent iron (ferric ion) in cytochrome oxidase of the mitochondria. The tying up of these oxidases with cyanide (cytochrome-oxidase CN complex) is readily reversible, and the enzyme present in the mitochondria, rhodinase, can transfer the sulfur from thiosulfate to cyanide to form thiocyanate. This restores proper oxidative metabolism by releasing the cytochrome oxidases.

The syptoms of poisoning are quite different from those seen in carbon monoxide. Cyanide is a true metabolic poison, and is lethal well before the hemoglobin of the blood is touched. Cyanosis, dizziness, headache, convulsions, are characteristic.

As with several others of the volatile poisons, cyanide exposure can be easily spotted from the odor. The person's breath is unmistakably the smell of bitter almonds. The treatment of cyanide poisoning is effective and will almost always save a victim if his heart still beats. It consists of two stages; first to free the oxidase system, and second to dispose of the cyanide ion.

There is a marvelous store of potential ferric ion in the blood, namely hemoglobin. In all normal forms, hemoglobin, oxyhemoglobin, and carboxyhemoglobin (Hb, O2Hb, COHb) the iron is in the ferrous state. However, it can be readily oxidized to the useless form containing ferric ion, called methemoglobin. But ferric ion, remember, is a tight binder of cyanide ion, and the concentration gradients found by the generation of methemoglobin are favorable for the robbing of the cytochrome systems of their sequestered cyanide ion.

(1) inject, i.v., a half gram of sodium nitrite in 15 ml of water over 4 minutes, to generate a lot of methemoglobin to free the body's needed oxidase systems from the cyanide ion.

The cyanide ion can be disposed of, separately, by its conversion to thiocyanate using the widely distributed enzyme rhodinase. A good source of sulfur is thiosulfate. This converts the toxic CN- ion to the relatively harmless SCN- ion which is easily excreted.

(2) then inject 12.5 g sodium thiosulfate in 50 ml water over 10 minutes.

Rebound may occur as there is a thiocyanate oxidase in the body which may reverse the latter reaction. This may need to be repeated. Also, remember, that the formation of methemoglobin also robs the body of oxygen-carrying capacity, and the administration of oxygen gas may be needed.

BARBITURATES

Another of the most frequently encountered emergency toxicology problems is the barbiturate overdose. The synergistic interaction of alcohol and barbiturates is widely appreciated, but still this combination is regularly encountered in both emergency and in the coroner's laboratory. At lower levels the barbiturates provide a depression of sensory function, with sedation but without analgesia. They can be anticonvulsants. At higher levels, the motor functions are also depressed, leading to both anesthesia and sleep. Death is usually due to respiratory failure.

The blood levels of barbiturates in toxicology cases are sufficiently high that analyses may be made without any concentration step. In general, the blood is replaced with water, and an acceptable analysis can be done spectrophotometrically. In the same analysis, two other acidic drugs can be detected. It is well to be aware of all three classes. Salicylic acid is a strong acid, and is the immediate metabolite of acetylsalicylic acid, or aspirin. And dilantin is a weak acid, weaker even than the barbiturates.

The analytical scheme can be outlined as follows:

Make the blood acidic. This precipitates protein and allows extraction of drugs into a solvent such as $CHCl_3$	All acidic drugs are in the organic phase.
	Aqueous has nothing $CHCl_3$ has everything
Extract the $CHCl_3$ layer with aqueous pH 7.5 buffer	Aqueous has salicylate $CHCl_3$ has the rest
Extract the $CHCl_3$ layer with aqueous pH 10 (borate) buffer	Aqueous has the barbs $CHCl_3$ has the dilantin
Extract the $CHCl_3$ layer with aqueous 0.5 N NaOH	Aq. has the dilantin $CHCl_3$ has nothing

Nothing is quite that simple. If there is a high concentration of salicylic acid, the simple extraction with pH 7.5 buffer may not remove all, and the salicylates will spill over into the borate extraction. Two pH 7.5 extracts may be needed. Also, at pH 10 some potential dilantin may be extracted, compromising the quantitative accuracy of the pH 13.5 extraction.

And with the barbiturates, one has the rare analytical situation that one can say how much drug is present, before one says just what drug is present. The extract made in the above scheme, if the volume of pH 10 buffer used is the same as the volume of blood originally employed, can be readily read in the UV to give a measure of barbiturate level as determined by the intensity of the peak at 240 nm. All barbs give just about the same peak and just about the same coefficient of absorption.

Acidification removes this absorption, allowing a baseline that permits quantitation. And from the acidified solution, the barb may be reextracted for subsequent GC or HPLC analysis identifying the actual species involved.

Urine analysis of barbiturates is usually very complex and disappointing. Veronal (diethylbarbital) is excreted largely unchanged, and phenobarbital is excreted partially unchanged, but most are extensively metabolized, and urine analysis is rarely worth the effort. Blood is the thing to go after.

SALICYLATE

The scheme given above allows the isolation of salicylic acid, which is the first and major metabolite of aspirin. The aqueous extract obtained by the pH 7.5 phosphate buffer, can be read spectrophotometrically, and a conversion to the protonated form with a drop of conc. HCl will complete spectral identification. Some people carry about an immense quantity of aspirin in the alleviation of the pains of arthritis, for example, and levels of 30 mg% may be found in therapeutic situations. But this level is suspect, and can represent a toxic situation as well.

NORMAL IONS FROM BLOOD AND URINE

There are standard clinical assays for the expected ions to be found in blood and urine, such as K^+, Na^+, and Li^+ in cases of lithium therapy. Some of these are obtained by atomic absorption, others by emission photometry. But they are rarely involved in toxicology problems. The heavier metals represent quite another matter.

HEAVY METALS

Almost all heavy metal ions are poisonous, and many of them do terrible things to the kidneys. But there are some urine tests that can be used as screening tests in urine for the presence of metallic cations. And they may be used directly on urine, without any isolation or concentration.

The Marsh test is highly sensitive for arsenic and antimony. Arsenic is a famous poison in the literature of murder and mayhem, and indeed, industrial exposure has been a environmental problem. But less known, is the gaseous hydride arsine which poisons through hemolysis and the resulting anemia and jaundice. The boiling of a urine specimen in dilute sulfuric acid and zinc produces the gas arsine (AsH_3) which on passing through a hot glass tube redeposits the metal, arsenic, as a mirror. There are a number of ways in which this mirror can be dissolved (nitric acid, hypochlorite) to identify it as coming from arsenic, or from the chemically similar antimony.

The Gutzeit test is similar, and is considered specific for arsenic. The urine sample, plus sulfuric acid and zinc, is heated, and the evolved vapors (containing the hydride arsine) passed through a piece of filter paper wetted with acidified silver nitrate. A bright yellow color is an exquisitely sensitive test for arsenic.

The Reinsch test is run within the urine itself. The urine is boiled after acidification with HCl, and several strips of shiny copper metal are dropped in. If arsenic is present, a gray coat will form on the copper. Variations of this test can be used for antimony, bismuth, and mercury. But care must be taken, as the anions of sulfur, selenium, and tellurium can get in here to give color changes.

Lead poisoning can be difficult both for diagnosis and for analysis. Dithizone (diphenyl dithiocarbazone) will extract cations such as lead and mercury, allowing their analysis by atomic absorption. Lead poisoning can be encountered amongst gasoline sniffers, and the quantities being poured into our environment from the broad use of leaded gasoline are very large indeed. The disease has been called plumbism, or saturnism. It has been implicated in the medical history of ancient Rome, with the use of lead both in the water system pipes and possibly in the treatment of wines. And in our current world, the syndrome called pica, the craving for, and compulsive eating of, small debris about us, has led children into consuming the lead paints of their cribs, and of course there are the pencils chewers.

Mercury poisoning is a painful and distressing process. Following acute exposure, there is an intense pain about the mouth and gut, with extensive vomiting and, if the poison has not been discharged, bloody diarrhea including portions of the intestinal mucosa. This usually leads to shock, and death in a few painful hours through circulatory collapse. Following lower, chronic levels, the kidney is the target organ leading to a generalized anuria.

There was a big flap over mercury poisoning and the problems of swordfish consumption several years ago. It was found that most of the fresh fish found off the coast of California had large measures of mercury in them. But this proved to be a on-going problem probably stemming from the use of mercury metal in the gold-extracting processes in the gold-rush years of a century ago in the state. Deposited metal mercury was converted to an organic form by bacterial action, and this was indeed picked up by marine life. It has been going on for a hundred years. And there is the tragedy known as Minamata disease. This was a situation that occurred in the 1960's, in which there was a spreading paralysis that occurred around this Japanese port city. It had led to many deaths, and was shown to be due to the discharge of mercury-containing wastes into the bay by a chemical company. The fish gathered the wastes into them, and when eaten, poisoned the eater.

THIN LAYER CHROMATOGRAPHY

One of the most powerful tools available to the analytical chemist is the technique of thin layer chromatography. It is most easily discussed in sections devoted to sample preparation, application to the plate, the choice of plates, the choice of solvents, the visualization, and the interpretation of the separations achieved.

Sample Preparation: Most TLC analyses employ a concentration of a biological sample approximately 1000 fold. With a sample of blood or urine, a fluid sample of 5 ml may well be reduced to a smudge that will be taken up in 5 microliters of solvent, all of which is applied to the plate. With relatively pure drugs, an effort is made to apply between a tenth and ten micrograms to the plate, depending upon the sensitivity of the developing reagent. Water is a complication with most TLC systems, so a considerable effort is made to keep the organic phases dry. One rarely wants to use the best solvent possible, but rather the worst solvent, one just good enough to do the job. This would minimize the impurity inventory of the drug extract.

With actual extracts from biological samples, one often encounters a gummy smudge that is spread over several square centimeters of the bottom of a evaporation tube. An additional rinse will bring this more to the bottom, but there is always some residue left up the sides of the tube. This is dissolved in a drop or so of the best solvent possible (the worst solvent was used in making the initial extract, and here one wants to make a concentrated solution of everything extracted), and shaken to dissolve as much as possible into this drop. This application solvent must, however, be volatile, so as not to interfere with the subsequent chromatography. Do not use DMSO, for example. And even with diligence in trying to get everything, one will end up with only about half of what has been extracted. This means that there is always enough material for a second, a confirmatory TLC analysis.

Application to the Plate: Some form of a micro-spotter must be used to transfer the isolated and reconstituted sample from the evaporation tube to the plate. Some people use capillaries, like melting point tubes, which are perhaps 2 mm in inside diameter, and will take up several microliters of solution. But with such a transfer technique, one usually gets a spot on the chromatographic plate that is a quarter of an inch in diameter. The application of the same volume of material with a micro-capillary, using a dozen touches of a smaller applicator, might give a spot of a fourth this diameter. The smaller the spot, the greater the potential resolution of mixtures as things progress up the plate. But if the spot is too concentrated, there can be a high rate of diffusion as soon as the solvent hits the spot, wasting much effort.

Using a commercial silica gel plate, on a glass backing, the site of spot application and a sample identification can be written directly on the silica with a soft pencil. A horizontal line is drawn parallel to the end of the plate, but far enough away

so that the developing solvent will not touch it. This will avoid dissolving the applied spots into the solvent reservoir. Small vertical lines then can mark the actual spotting locations. There should be little difficulty in putting seven to a 5 cm wide plate, and perhaps 30 to a 20 cm plate. Do not use a felt pen unless you want to separate the dyes of the pen's ink!

If the plate contains a fluorescent indicator, and UV absorption is to be used as a locating tool after chromatography, then a check can be be made before chromatography to verify that a sufficient amount of sample has been applied to the plate. If you can't see it before separation, you will never see it afterwards.

The Choice of Plate: A large proportion of TLC chromatography today employs plates containing Silica Gel as the stationary phase. A typical thickness is 250 microns, or a fourth of a millimeter. The plates can be made of glass (most common), plastic (mylar) or aluminum. A binder is used, and often, a fluorescent indicator is also mixed in, allowing a fluorescent activation with a short-wave UV light (a mineral light) that will reveal aromatic compounds. The silica is attached very strongly, and will withstand considerable physical impact. However, solvent systems with a goodly percentage of water or very strong base will dissolve the binder and cannot be used.

The two other major TLC plate types are alumina, and cellulose. It is often said that plates should be activated before use, but this is only true with alumina types. A reasonable precaution with silica and cellulose is to keep them in a relatively dry environment, perhaps in a desiccator for a few hours before use, or perhaps a brief heating in a warm oven. But there is little change of performance seen with slight moisture changes. With alumina however, there is a real degree of activation that reflects the degree of heating and/or drying applied before use. This will change the apparent acidity of the alumina, and therefore the nature of the separation achieved.

Choice of solvents. The art of TLC is the choice of the solvent. The stationary phase in this partitioning is relatively inflexible. With silica (or alumina) it is strongly acidic and quite polar, and with cellulose, it is neutral but quite polar. The choice of solvent is dictated by the nature of the materials to be separated. They will be partitioned between (say, with silica) a polar stationary phase, and the solvent moving phase. If the mixture is quite polar, then a polar moving phase will be needed to compete with the silica. As the mixture to be resolved becomes less and less polar, the moving phase must do the same. Methanol, methylene chloride, and hexane represent a useful set of solvents to initiate exploration of separations. Start with methylene chloride, and if the materials move too slowly (too tightly associated with the silica) then move to methylene chloride and methanol mixtures. Similarly, if the mixture of components moves too quickly, decrease the moving phase's polarity by trying a mixture of methylene chloride and hexane. Remember that methanol and hexane form a two phase system and cannot be used together. Also, the higher the boiling points of the solvents (in general), the less the diffusion of the spots, but the longer a chromatograph will take, both to develop and to dry afterwards. Also, the more polar the moving phase, the more slowly it will advance up the plate.

Another consideration must be noted with the attempted separation of basic mixtures. The silica gel stationary phase is not only highly polar, but is acidic as well. The term "silica" is actually silicon dioxide, SiO_2, and is seen in the form of amethyst and sand; it is neutral. The term "silica gel" refers to a precipitated form of silicic acid, a hydrated form of silica with a formula of approximately H_2SiO_3; it is acidic. So, with the application of basic organics to a silica gel plate, salts are formed, and even quite polar solvents may be ineffective. A useful change is to add a base to the moving phase as well, thus neutralizing the silica gel acidity. Typically, ammonia or a simple alkyl amine is a minor but important component of the moving phase.

When the plate is spotted and the solvent has been chosen, the TLC is ready to run. Classically, many precautions are called for. The chamber should be air-tight, the solvent should have had the opportunity to vaporize and equilibrate. The plate should have been in this vapor environment allowing it, too, to equilibrate. The temperature should be constant. And on and on. In practice, we usually just slap the plate into a container containing the solvent at the bottom, cover it with a watchglass or foil, and let it go. Capillary action within the interstices of the stationary phase conducts the solvent up the plate, and the extent of development is easily seen from outside the developing tank. The further the development, the slower the development, due both to evaporation of the solvent and to gravity. As the leading edge of the solvent (the front) moves more slowly due to evaporation, the spots on the plate may still be in a portion of the system where there is rapid movement of the solvents. Thus, their position relative to the front (this relative position is call the Rf which is:

$$Rf = \frac{\text{distance of spot center from origin}}{\text{distance of solvent front from origin}} \times 100$$

and which is often used to characterize a drug in a given TLC system) may be changing. When the chromatogram is completed (often one can stop at the half-way point, getting about as good a separation as will be had) and the unused half of the plate can be used for a second run, by simply inverting and respotting. The extent of the solvent run (the front) should be marked in some way as soon as the plate is removed from the developing tank.

Visualization. Just as important as achieving the separation is seeing what you have done. This visualization step may employ either non-destructive or destructive procedures. If any further analysis is to be done of the separated materials, only non-destructive visualization techniques can be used.

Ultra-violet light. Silica gel plates are commercially available that contain within the stationary phase a fluorescing indicator (such as zinc cadmium sulfide), which will emit a pale yellow-green light when activated by a short wave-length U.V. lamp. Such plates are marked with a code that includes the number 254. If the components that are to be visualized contain conjugate double bonds, or aromatic rings, these will absorb this fluorescence and will appear as dark spots on a pale yellow-green field. A common convention is to mark them lightly on either side (in parentheses) with a pencil. Then, exposure of the plate to a long wave-length U.V. light

(365 nm) can reveal additional information. This long wavelength will not excite the phosphor within the silica gel, but it will cause many types of organic compounds to fluoresce in their own right. These, then, will show up as light-emitting spots, with characteristic colors, on a dark background. Such spots are often outlined from above and below, with a soft pencil. The reasons for these markings are to make a recallable record for, at a later time, some obscuring spray may be used which will hide any subtle U.V. indicators, or some components may diffuse or even evaporate from the plate entirely on standing in the open air. Of course, if one of the solvent components is U.V. absorbing (such as benzene) the plate must be thoroughly dry before viewing.

Color tests. The development of colors for the visualization of spots on a TLC plate can be either non- destructive or destructive. With alkaloids and with most basic drugs, there is the development of an intense purple color when the plate is sprayed with iodoplatinate. The spots fade (or more likely, the plate slowly develops to an intensity to match the spots) but the process leaves the base chemically intact. It can be removed, the salt disrupted with sulfite, and the drug removed for additional analysis (such as GC). Other common color developers, such as ninhydrin for amines and amino acids, or PDAB (para-dimethylaminobenzaldehyde for pyrroles such as LSD or indoles) chemically react with the spot, and are thus destructive. Again, when a base has been used in the moving phase (such as ammonia which will give a strongly colored salt with iodoplatinate) the plate must be completely dry (odor-free) before spraying. The spraying technique itself has some aspects of an art-form. A light polka-dot spatter is prone to produce spots of developer that vie with spots of chemical to be developed. And, on the other hand, a drenching can obscure everything and even move the spots with the flowing developer. Until the skill is in hand, consider spotting the mixture to be assayed on a scrap of TLC plate, letting dry without any development, and using it as a trial spray target. Also, the excess spray can be quite corrosive if breathed, and can contaminate a sizable area. A spray box or well ventilated hood is needed.

Universal sprays. These are excellent for the total organic composition of the mixture that has been separated, but are totally destructive. Spraying a plate with conc. sulfuric acid and subsequent heating, or spraying with a 1% potassium permanganate solution in dilute base, or placement of the plate in an iodine gas chamber, then air-drying and possibly starch spraying – all – are excellent detectors of organics on a TLC plate. Remember, though, that cellulose is an organic as well, and may not survive a universal spray.

Interpretation. I have heard it said by experts in court, under oath, that the Rf (see above) of a drug is one of the most dependable and constant physical property it can have. And that if an unknown has the same Rf as a reference drug, it is proof that the unknown is in fact the reference drug. Hogwash.

Just how constant is the Rf of a drug? It can vary over a wide range for a large number of reasons. First, just how accurately can one even measure the Rf? The origin where the spot was applied is clearly marked, but the front can be a little more diffuse. With the more volatile solvents, you can see it disappearing after taking the

plate out of the developing tank, even while you are looking around for a pencil to mark it. And there is a phenomenon known as the false front. When mixtures of solvents are used as the moving phase, there is often a tendency for the most volatile to evaporate preferentially from the plate during the actual chromatographic run, so that there is a fractionation of solvent along the length of the plate. Then, when the plate is removed from the tank, there is an apparent true solvent front, and a bit below as you watch, there appears a second solvent front (an unmistakable change of pace in the drying of the plate) which really serves as the de facto limit for the movement of any component in the mixture to be chromatographed. Spots rarely appear in this twilight area between the two fronts. Which is to be used? And to complicate things further, the center of the spot enters into the calculation of the Rf, and where is the center if the spot has the shape of a bartlett pear, or tails half the way back to the origin?

So the measurement of the apparent Rf can only be estimated to a few percent (a position of two or three millimeters on a run of maybe ten centimeters). So an inventory of perhaps a total of thirty or forty Rf values must be shared by some tens of thousands of drugs. So much for the argument that the Rf will define the drug.

But the displayed Rf can vary from run to run, and even within a single run. Factors such as slight variations in solvent composition, inconsistencies in moisture content of the plate, vapor equilibration of the tank and the plate itself, extent of solvent migration on the plate, edge effects of the solid phase thickness on the machined sides of the plate, even production variations on the silica gel sizing and thickness itself – all can affect the observed Rf. A careful chromatographer will flank his unknown sample with reference drug at about the same quantity, and observe the chromatographic behavior of known and unknown. If possible, alternate the known and the unknown clear across the plate, so that subtleties in both color and motion will be apparent.

The tailing of a spot is always a source of concern. This is the term given to a teardrop-shaped spot that shows some retreating trace usually towards the origin, or point of application. Many causes can be looked for: there may be a continuum of components, such as a homologous series from some natural source; there may be an association with the stationary phase that is slow to disrupt; there may be a partition coefficient that is a function of concentration; there may simply be far too much material on the plate and there may be far too few binding sites of the silica gel. This latter situation is commonly called overloading. Usually a drastic change in solvent composition (from basic to acidic, for example) can remedy the situation, or in the case of overloading, a smaller sample should be spotted.

Two-dimensional TLC. A powerful technique in TLC chromatography comes from the early days of paper chromatography. This involves the placement of a single spot in one corner of a square TLC plate, and the development of this spot the length of the plate with a given solvent system. Then after complete drying, the mixture that was in the original spot is spread along a line on one side of the plate. Then, with a plate rotation of 90 degrees, this line can become a new origin, and a repeat of the

entire chromatographic process made, with a different solvent system. The result will be a two-dimensional spread of the components of the original mixture which can, with component identification, serve as an atlas of a frequently analyzed mixture. Spots containing reference standards can be co-chromatographed by being placed in the appropriate corner , where they will each be subjected to only one of the solvent combinations. The use of two-dimensional chromatography with the same solvent being used in each dimension, can resolve the identity of the tailing questions mentioned above.

GAS CHROMATOGRAPHY

A major analytical tool in the criminalist's lab, and in the clinical chemist's lab as well, is the gas chromatograph. It has been mentioned in the area of alcohol analysis, but in fact it is the first instrument that an analyst will turn to, to start an analysis for the presence of a drug in an unknown sample. The only requirement of a drug to allow GC to be used, is that it be sufficiently volatile. And 95% of all drugs are sufficiently volatile.

The procedure is straight-forward. A solution containing the material to be analyzed is vaporized in a small chamber, and the components of this vaporization are swept into a column that has as the stationary phase a liquid on a solid matrix (see earlier under the general discussion on chromatography in Lecture #8) and a moving phase simply a hot gas. At the end of the column, the components are detected as they emerge, and the results of this detection are recorded by some form of a print-out. Two of the major variables in GC work, are the nature of the column (both the liquid substrate and the geometry of its deposition) and the nature of the detector.

COLUMN GEOMETRY

Originally all columns were packed; this was the standard of the industry. A metal or a glass tube, from an eight inch internal diameter to as much as an inch (for preparative separations), was filled with a finely pulverized solid support that was uniformly coated with a liquid material. The materials used as the stationary liquid have been most variable, from hydrocarbons to silicones to rubber and related polymeric chemicals. The boiling point had to be high enough that there would be an acceptably small amount of bleeding from the column to the detector.

The concept of a capillary column was worked on in the early days of GC, but only recently has become well-enough engineered to be practical. Originally, glass was the column material, and the packing was, rather than on a solid matrix, simply coated on the inside surface of the capillary. They would bubble closed easily, they would break without warning, and they would quickly clog up at the injection end with the charred gook that is generated by the flash volatilization. Two developments have made this technique now a routine procedure. The manufacturing of capillaries out of quartz has made them virtually immune to accidental fracture. They may be dropped and banged about with surprising casualness. A problem with front-end contamination is simply solved by breaking off a few inches and, voila, one has a new column.

A second development has been the ability of bonding the liquid stationary phase to the inner surface of the capillary, thus making it easily cleaned and relatively unaffected by repeated injections of solvent. A fringe benefit of the capillary now being readily available is that the use of a mass spectrograph as a detector is easily achieved.

It is obvious that the nature of a GC instrument and a Mass Spectrograph (MS) are quite opposite in their requirements. A GC uses gas under pressure, and by definition, the detector is fed by a stream of gas that is at least at atmospheric pressure. The MS, on the other hand, can only work in a high vacuum. So, the marriage of the two required that all the carrier gas be removed from the emerging stream from the GC before being introduced into the MS. And at a temperature that precluded the condensation of the components being separated from one another. The very small volume of gas that is intrinsic to the capillary column made this separation possible, and the GCMS is rapidly becoming a routine laboratory instrument.

GAS CHROMATOGRAPHIC DETECTORS.

The Thermocouple Detector (TC): The earliest detectors called upon heat conductivity for the analysis of emerging peaks. The sample that was swept out of the tail-end of the column passed through a detector that determined the heat conductivity of the emergent gas composition relative to a control standard. When an unusually low conductivity gas was used as a carrier gas (hydrogen or helium) then the presence of an organic compound in the out-flow created quite an unbalance in the detector. And this unbalance was recorded as an emerging peak. This procedure had a low sensitivity, but was completely non-destructive and formed the basis for the first preparatory chromatographs.

The Flame-ionization Detector (FID): This device was developed in the early 60's, and is still a mainstay in the analytical field. In this system, the emerging sample is burned in the detector, and the conducting plasma that is the result forms the basis of the recorded signal. As all things burn to an approximately similar composition, all things give similar responses. This is called the universal detector as the response areas are largely independent of the composition of the emerging peak. In principle, air and water give no response, in contrast to the thermal detector above.

The Electron-capture Detector (EC): This is an incredibly sensitive detector that was developed twenty years ago, and served as a major tool in the development of analyses with insecticides. It is based on the capability of compounds (peaks emerging from the chromatograph) to absorb low-energy electrons as might be found from tritium (H-3) or Ni-63. Those materials which could absorb a "soft" electron gave very high responses (such as polychlorinated things) and those which did not absorb these electrons were without response (hydrocarbon solvents). The results were non-linear, probably destructive, and extremely dependent upon the structure of the molecule being assayed, but the sensitivity obtained was unprecedented.

The range of responses is shown in the following table, based on chlorobenzene equaling 1.0.

COMPOUNDS OR CLASSES	EC SENSITIVITY:
Aliphatic or aromatic hydrocarbons	0.01 to 0.1
Alcohols, ketones, aldehydes, amines, nitriles	0.01 to 1.0
Monochloro- or trifluorocompounds	0.1 to 10
Ether, oxalates, stilbenes, azo-benzene, acetophenone	1 to 10
Dichloro or monobromo compounds	10 to 100
Trichloro- dibromo- monoiodo-hexafluoro- or mononitro-compounds; benzophenone, benzaldehyde, azulene	100 to 10,000
Tetrachloro- tribromo-, diiodo- or dinitrocompounds; quinones, pyruvates, diacetylcompounds	1000 to 10,000

There can be as much as a million-to-one ratio of responsiveness from one compound to another. EC is now losing popularity in favor of the NP detector and the mass-selective detector.

The Nitrogen-phosphorus Detector (NP), also called the Thermionic Emission Detector (TED): For drug work, this is probably the hottest thing available at the moment. Since most drugs contain nitrogen, and most solvent systems for GC work can be made to avoid nitrogen, this detector is the most sensitive and noise-free that is currently available to the analytical chemist. The detector is a flame-like system, but it has a small bead of an alkali-metal salt near the flame, which promotes an extraordinary sensitivity to emerging compounds that have nitrogen (or phosphorus) in their make-up. With nitrogen the response is 75,000 times, and with phosphorus, 35,000 times more than that of carbon.

The Redox Chemiluminescent Detector is a recent addition to the stable. In this, the effluent from the column is exposed to NO_2 or nitric acid, and if oxidation occurs, the resulting NO is separated, and independently reoxidized to NO_2 with ozone. The reaction gives off light, which can be detected and recorded. The responses are linear, and with the appropriate choice of catalyst (gold or platinum) and detector temperature (up to 350 degrees) an extraordinary signal to noise ratio can be had.

The Mass-selective Detector (MS): Within the next few years you will hear more and more about the use of mass-spectrography in conjunction with GC, and the term

GCMS will become commonplace. This procedure employs a mass spectrograph (to be discussed in some detail in lecture #16) as a sensitive detector for the GC. As the detector can be set for specific mass units as well as for total ion current, an additional physical property is obtained in the identification of an emerging peak (a mass fragment value as well as retention time).

The Photoionization Detector (PID): The action of this detector is based upon the ionization of an organic compound by a UV lamp with a variable energy, allowing freedom of response from water or carrier gases. The detector has a wide range of linearity, is about 10 to 50 times more sensitive than the FID, and can respond to several inorganic compounds. However, compounds must have ionization potentials less than the energy of the UV source (less than 10.2 eV).

PREPARATIVE GAS CHROMATOGRAPHY

A dream that has always bedeviled the chemist is the possible extension of the efficiency of the analytical procedures of chromatography to the possible employment of those techniques for the isolation of tangible quantities of material. So, every analytical technique (with its micron and millimeter size) has been reengineered to a production technique (with its inch and foot size). None have been completely successful. In the GC area, scaling up to a column of about a half inch diameter has allowed the separation of multi-milligram quantities of components of mixtures, but with a considerable loss of resolution. The high resolution of the capillary cannot be exploited in a preparative system, at least within today's technology. And an additional point to remember, the use of a system for the isolation of a product must require a non-destructive detector. Originally the thermal conductivity detector was required, but more recently, splitting systems allow for the sensitivity of FID or NP detection with the loss of only a minor portion of the sample.

TEMPERATURE PROGRAMMING

Originally, all GC analyses were performed at a single oven temperature, or isothermally. The emerging peaks formed an easily recognized pattern, each having a half-width that was proportional to the time required for emergence from the column. But with the early instrumentation, the requirements of stability and reproducibility were such that all instrument parameters had to be held constant. But with the development of better sensors and electronic control circuitry, the values of temperature programming are now routine. If the temperature of the oven of the column can be reproducibly raised during the course of a chromatographic run, then the higher boiling (or more firmly absorbed) samples act as if they were held on the first portion of the column until their effective movement temperature had been achieved. The result is a chromatogram with no progressive broadening of peaks as the run progresses. And with the sharpness and high resolution of the capillary system, the effective resolution is most impressive.

INTEGRATION

A necessary part of analysis is quantitation. A peak emerges from the GC. How much material is there? This requires the measurement of quantity, and the establishment of standards and controls. The quantitative aspect depends upon the linearity of the detector and the intrinsic responsiveness of the compound being assayed.

If a detector is linear (i.e., gives a response that is proportional to the amount of the sample being detected) then one need only measure the integrated output of that detector. If it is plotted on a sheet of graph paper, then the area under that plot is proportional to the amount of material that reached the detector. Twice the area, twice the amount. In the early days before dependable electronic integrators, we would actually make a Xerox copy of the output copy, cut out the peak, and weigh it on an analytical balance, to get proportionate amounts. And with this same linear detector, if the half-widths of the peak of the unknown and the peak of the reference were the same, simple triangulation would allow the measure of the peak height as being an accurate measure proportional to the area, and thus to the quantity.

Each of these approaches is compromised by the need of knowing where the baseline is under the peak. The area requires completing the peak with a baseline, and the halfwidth and the height both require a baseline from which to measure. But the baseline cannot be there, because the peak was there, and only one line was being drawn. No problem if there happened to be a mile of flat baseline both before and after the desired peak – there was probably a flat line under the peak too if it could only be seen. But all too often the peak is on a sloping baseline rich with small bumps and warts, and you simply have to guess and use good judgment. And your accuracy reflects this judgment.

An equally important parameter is that of equating peak height or area to an actual value. All of the above provided only proportionate amounts. The actual absolute amounts requires the generation of a standard curve. The is an inescapable point of highest importance in any quantitative analytical scheme.

STANDARD CURVE

Two terms are often interchanged in analytical work; standards and controls. They are absolutely distinct and different.

Standards are solutions of known drug that are made up in water, or in the medium to be assayed, that have an absolutely certain concentration. They have been made so. If you have a set of standards, of several concentrations, and you submit them to your assay, you will get a set of values (areas under the curve, peak heights, summed detector responses, it doesn't matter what) that will vary with the quantity of reference material that had been put in each standard solution. The diagrammatic presentation of detector response (usually on the Y-axis) and the known quantity of material present (plotted on the X-axis) produces what is called a standard curve. The fitting of a best-fit line to the experimental points provides a conversion so that any

unknown, finding the detector response, allows the immediate calculation of the quantity present.

If, however, some of the specimen had been lost, or if a wasteful injection into the machine had been made, or if the extraction phases had not separated completely, or if there had been carry-over from one part of the extraction to another, it is possible that the amount reported might wrong to a degree that could not be estimated. To correct for this, a frequently employed feature is the internal standard.

Knowing the properties of the drug to be assayed for, it is possible to construct a similar compound, similar in partition and extraction rather than in pharmacology, which would follow the desired drug through all the isolation and analysis steps, but be distinct in two ways: It would be seeable separately in the final analysis, and it would not be present in the original sample to be analyzed. Then, instead of determining peak heights (or areas) as absolutes one can determine ratios of heights (or areas) between the unknown and the internal standard. This easy maneuver corrects automatically for mechanical losses, but it is no better than the quality of the design of the standard itself.

QUALITY CONTROL SAMPLES

Controls are quite a different thing. The standard tells you what the exact value should be. It gives the number. The control gives assurance that the value is exact. It verifies the entire analysis. The common way of maintaining quality control, is to prepare a number of true, biological samples, that have been assayed and have been found to have acceptable values. Say, draw a liter of blood from a person who is moderately drunk, and subdivide this blood into 200 5 ml tubes, and put one of these into each day's analyses. Here, the value is known only by statistics, but it serves as a consistency control to the daily standard curve. If its analysis is OK, this is a check both on the standards and on the overall analysis. But if it is wrong, if it is outside of an acceptable limit of error, then the analysis is "out of control" and everything stops. The standards may well change from day to day, and be quite capricious, but the control, using each day's standards for that day, cannot vary. Sadly, many laboratories use carefully checked standards and standard curves, but ignore the quality control. Both are essential.

HIGH PRESSURE (PERFORMANCE?) LIQUID CHROMATOGRAPHY (HPLC)

HPLC has emerged as a regularly used instrument in the analytic laboratory. It is a modern-day version of liquid chromatography, using smaller diameter columns, finer particles, and the use of a high pressure pump to achieve separations in a reasonable period of time. The entire system, from beginning solvent reservoir to final detector, is self-contained, and is designed to be free from any dead volume which would destroy resolution. A dampened pump delivers solvent to a column at pressures of up to 4000 lbs, and the column is packed with a material of a size that allows solvent to flow at a rate of one or two ml/min. The sample is applied to the column through a loop that allows application of the unknown sample without

interrupting the solvent flow. The column itself can be silica gel, imitating the direct phase nature of TLC with the conflict of polarities dictating the order of emergence of the components of a mixture. But here, the polarities can be easily reversed. It is easy to involve the acidic functions of the polar silica gel in chemical reaction through a silyl intermediate to form a non-polar surface. Instead of the polar -OH groups sticking out and competing with the moving solvent in the partition coefficient dance, the group has become chemically modified to an aliphatic hydrocarbon

$$(\text{-OSi(CH}_2)_2\text{CH}_2\text{CH}_2\text{CH}_2\text{-etc-CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_3$$

and thus is totally non-polar. These are commonly called C-8 if the alkyl group is octyl, or C-18 with an octadecyl group. Now the moving phase must compete in an opposite manner, and unexpected separations are possible. Intermediate polarities are available, with silyl attachments of phenyl groups, or cyano groups, to the otherwise acidic and polar silicic acid groups.

Many types of detectors are available to HPLC systems, the only requirements being that the void volumes must be kept small for good resolution. The U.V. detector is a mainstay, and systems are available that are not only tunable in frequency (allowing a maximum response for a desired compound) but can be spectrum-swept to give the complete spectrum of any emerging compound. Fluorescence detectors are routine, employing cavity cells that are constructed to have a dead volume of only a few microliters. Electrochemical detectors can respond to the voltage needed to oxidize the emerging chemical. Radiochemical detectors can locate peaks that contain radioactivity. And there are many procedures that employ post-column derivitization, calling upon a color reaction to provide the information that will allow detection of a wanted chemical. These chromatographic systems have achieved considerable popularity in that they may be used, with the proper components, with urine or even blood samples without any preliminary clean-up. Thus an analysis may be run with neither technical knowledge nor laboratory sophistication on the part of the analyst.

PHYSICAL AND CHEMICAL ATOMIC WEIGHTS

Each year I say that I am going to spend a little more time talking about the concepts of physical and atomic weight, because each year I discover that a large percentage of my students have never been clear in this matter. And each year I try a new approach in this presentation, and there are always a few who don't get the music of what I am saying. I want EVERYONE to get these concepts, since without them there will never be an understanding of mass spectroscopy, nor of NMR, nor of radioactivity nor nuclear chemistry. Each year I start with yet a different approach, and still there are those who say, yes, they understand, although they do not. Let me start this year with yet a different approach. For those who are at peace with all of this, be patient and go on to the next lecture. This is one of my four or so concepts that everyone in the class must get. I may even take the entire lecture on this one thing.

ATOMIC WEIGHT:

Maybe this is the best level at which to start. What is the atomic weight of an element? Here is a good point of departure for this diatribe on atomic weights.

THE WEIGHT OF AN ELEMENT HAS NOTHING TO DO WITH
THE WEIGHT OF AN ATOM.

Let us consider a very big bag filled with lots and lots of hydrogen atoms. For the moment let us dismiss the physical fact that they are doubled up in H_2's but simply assume that they are lone-riders and free-lancers all of which are simply H. This was chemical fact only 150 years ago, for it was only recently that the dimer structure of hydrogen, nitrogen, oxygen, etc., was appreciated. Almost all of these hydrogen atoms have a mass of about one, since they are structurally a single proton and a single electron. Any atom with a single proton and a single electron has a very well described mass, completely without variation (as long as there are no relativistic games in the territory). And this proton will have exactly the mass, 10 to the minus something-or-other grams per proton. In short, you have a gillion protons of mass such-and-such, so the total weight is a gillion times such-and-such, or so-and-so grams. And this would be the weight were there so many protons of so much weight. And the average weight is the EXACT weight divided by the EXACT number of hydrogen atoms.

If one takes as a reference point that an atom of the most common isotope of carbon, the mass-about-twelve isotope, is exactly 12, then the mass of any of these mass-about-one hydrogen atoms is exactly 1.0078252. In fact, the mass of every one of these mass-about-one isotopes is exactly 1.0078252.

However, there is a small complication in this picture, when you are dealing

with real hydrogen taken from the real world around you. Depending upon the source of the hydrogen you are looking at, one atom out of every few thousand happens to have a mass of about 2. This is "heavy" hydrogen, commonly called deuterium, and the mass of this mass-about-two hydrogen is exactly 2.0141022, again based on the arbitrary acceptance of the carbon atom with mass-about-twelve being exactly 12. And, as with the "normal" or "light" hydrogen, the mass of every one of these mass-about-two hydrogen atoms is exactly 2.0141022.

The frequency of encountering this occasional "heavy" hydrogen depends upon where you collect your hydrogen sample. In some samples it is common (one atom out of every 6410 atoms collected) and in some samples it is less common (one atom out of every 7520 atoms collected). These are the known extremes in natural occurrence. Thus, the average weight of all hydrogen atoms as they really occur in nature has this intrinsic variability, and the published value, 1.00797, can only be stated to 5 figures although the two components are known to seven places.

A very minor contributor is hydrogen with a mass of about three. This is the radioactive isotope tritium which was a very rare component of our environment before man-made radioactivity came upon the scene, and is now somewhat more common. Sadly, its half-life (12.26 years) is not short enough to stay abreast of its production and release to the environment. For collectors of trivia, its exact mass is 3.0160494.

So, when you look at hydrogen a milligram or a kilo at a time, the atomic weight is about 1.00797. This is the number that enters the calculations of stoichiometry. And when you look at hydrogen an atom at a time, the atomic weight is either 1.0078252 or 2.0141022. These are the numbers that enter calculations involving mass spectroscopy.

A term that is frequently used in mass spectroscopy is the nominal mass. With no exceptions, the nuclei of atoms are at almost even mass units, since they are made up of integer numbers of protons and or neutrons. Actually, these are not real protons and neutrons, but things very much like them at one time, which have lost little bits of mass in the process of getting scrunched together in a nucleus. So a mass of 31.972074 (that of ^{32}S) is said to have a nominal mass of 32. This is a matter of rounding up (or down for the light-mass isotopes) to the whole integer that represents the actual count of original particles that make up the nucleus. Atomic weights that are from the chemical world have no such neat rounding allowed, of course. And when one speaks of the most massive nucleus of hydrogen, for example, this would be ^{3}H. And if this is of a molecule, and all of the isotopic possibilities are being considered, the the most massive molecule of methane (CH_4) would be $^{13}C(^{2}H)_4$ or a nominal mass of 21 (considering only stable isotopes) or $^{14}C(^{3}H)_4$ or 26 (considering the unstable isotopes as well). The convention of preceeding the atom with the isotope nominal mass as superscript is currently used in writing. The term C-14 is stilled in speaking of these isotopes.

For reference purposes, here is a listing of the isotope masses and atomic weights of the elements most frequently found in drugs:

ELEMENT AS FOUND IN NATURE (chemical at. wts.)		ISOLATED ISOTOPES (physical at. wts.)	
H =	1.00797	^{1}H =	1.0078252
		^{2}H =	2.0141022
		^{3}H =	3.0160494 *
C =	12.01115	^{12}C =	12
		^{13}C =	13.0033554
		^{14}C =	14.0032419 *
N =	14.0067	^{14}N =	14.0030744
		^{15}N =	15.0001088
O =	15.9994	^{16}O =	15.9949149
		^{17}O =	16.9991322
		^{18}O =	17.9991598
F =	18.998405	^{19}F =	18.998405
S =	32.064	^{32}S =	31.972974
		^{33}S =	32.971460
		^{34}S =	33.967864
		^{35}S =	34.969034 *
		^{36}S =	35.96709
Cl =	35.453	^{35}Cl =	34.968854
		^{37}Cl =	36.965896
Br =	79.904	^{79}Br =	78.91835
		^{81}Br =	80.91634
I =	126.90447	^{127}I =	126.90447

* indicates an unstable isotope

THE GENERIC MASS SPECTROGRAPH

All of the above has been in preparation for a discussion of a mass spectrograph. Here I want to talk about the general idea of molecular separation; in the next lecture I will give finer details of the several ways in which it can be done.

In the simplest terms, an analysis of a molecule or of a mixture of molecules involves the generation or attachment of a charge to the molecule (or molecules, or fragments of molecules) and the subsequent determination of the mass of the thing

that carries the charge by deflecting it somehow. By knowing the value of the charge, one can get the mass of the thing by measuring the deflection.

All mass spectrographs (spectrometers, remember graph = write, meter = measure?) can be considered as a sequence of five parts. Input, ionization, focus, mass analysis, and detection.

INPUT: Samples may be introduced directly into the mass spectrograph. This is done by layering a reasonably pure sample on a probe or filled into a glass capillary. It is then inserted through a series of vacuum locks to get it close to the ionization source. There it is heated and volatilized (it has to have a usable vapor pressure). The sample requirement is a few micrograms.

Indirect inputting is now becoming the major procedure, to make the tool an ally to gas chromatography. However, this marriage was long cursed with the simple conflict – a gas chromatograph uses a carrier gas, and so operates at or above atmospheric pressure, and a mass spectrograph requires a very good vacuum. How does one free a fraction of a microgram of a test compound from the surrounding buckets of carrier gas? Helium (or hydrogen) was a natural choice as it has a low molecular weight and a high ionization potential. Its low momentum makes it easier to pump away from the stream of material emerging from the GC column, and the high ionization potential makes it less likely to be an associating ion in the subsequent analysis.

Two general types of separators are now used. The jet separator (Ryhage type) employs two small apertures in the line of gas flow, and requires a separate pump between these apertures to remove the helium. The higher momentum of the samples to be analyzed carries them (as a jet) through the second aperture. It is rather expensive, can plug up easily from column bleed, and also applies a slightly negative pressure to the GC column. However, it does not have limitations to either temperature or to specific types of chemicals as analytes. The Watson-Biemann type of jet separator employs a porous column between the apertures.

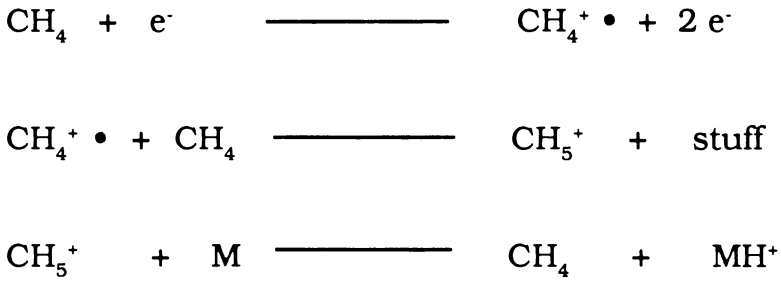
The membrane separators depend upon diffusion and permeability for the separation of a sample from the carrier gas. A silicon membrane a few microns thick is used. The carrier gas is insoluble in it, whereas the organic molecules are soluble. The process is dependent in a complex way upon temperature, as the solubility will decrease, but the diffusion efficiency will increase, with an increase of temperature. This technique is limited to organics that are soluble in the membrane, but it is inexpensive and easily replaced as needed.

Centrifugal procedures are known, but are rarely used. The use of capillary GC is a great asset, in that there is a very restricted quantity of carrier gas used. And with chemical ionization MS (see next lecture) the situation is better yet since hard vacuums are not needed, and the carrier gas can actually be the same as the ionizing gas.

IONIZATION: This is the process of converting the sample into mass units that carry a charge. This charge is usually positive, resulting from the loss of an electron or the presence of a closely associated proton.

Electron impact ionization (EI) is one of the most commonly used. The sample, now separated from the carrier gas in the case of GCMS, is impacted with electrons of about 70 volts energy which can knock bonding electrons out of the molecule which is being analyzed. When one electron of a two-electron bond is lost, the damaged molecule easily falls apart. The two fragments are different in that the remaining electron goes with one or the other. The fragment with the electron is now a radical and being uncharged plays no further role in the process. The fragment without the electron has a positive charge, and if stable can be swept into the focusing portion of the instrument. Certain fragments easily rearrange into stable configurations and are useful in structural determination. The use of lower voltage ionizing electrons gives less fragmentation, but with much less sensitivity.

Chemical ionization (CI) is achieved by first ionizing the carrier gas (or the gas surrounding the sample to be analyzed) with a low energy electron source. The ion-radical formed then abstracts a hydride from a second, unionized molecule and this species transfers a proton to the molecule being analyzed. With methane as the ionization gas, the following equations apply:



A large variety of other gases can be used; isobutane, ammonia, or even water have been employed. There is much less fracturing of the molecule due to the lower energies of ionization (perhaps 10 volts) and the parent peak (plus one mass unit for the proton, of course) is often the major component seen. This make CIMS especially valuable for determining molecular weights, for resolving mixtures, and for quantitative analysis. A compliment to chemical ionization is negative ion formation, where the use of certain carrier gases (such as nitrogen or nitrous oxide) can actually abstract a proton to give a negative ion. This area is still in its infancy.

FOCUS: The collection of particles, fragments of molecules or intact parent molecules that are carrying a charge are directed by a series of lenses which are simply metal plates with holes or slits, electrically charged with the same sign as the charged particles themselves. These are arranged to narrow the beam, thus increasing its

density, and directing it into the mass analyzer. They can also serve to remove any ions of the opposite polarity.

MASS ANALYSIS: This is the heart of the instrument, where separations are made on the basis of mass to charge ratio.

Magnetic deflection is the oldest method, and is still broadly used. The beam of ions is passed through a magnetic field, and as they all (or almost all) have the same charge, the deflection will reflect the momentum and thus the mass. The larger the mass, the less the deflection. These instruments can have a very broad mass range. But they are large, and expensive, and unstable, requiring continuous calibration.

Time of flight analysis is of limited sensitivity and selectivity, and is not often seen now. Here, the compounds to be analyzed are ionized in a short pulse (perhaps a microsecond) and are submitted to a carefully controlled accelerating potential. There is a fixed area for drifting between this accelerator and the detector, and the lighter the fragment, the sooner it will reach the detector. The mass of the fragment is measured as proportional to the time required to traverse the vacuum chamber.

Quadrapole analysis. Here I am out of my depth, but in general there is a balance of DC charge, and an oscillating RF field applied to four poles arranged parallel to one another and at the corners of a square. The beam is directed between these, and the combined DC and RF voltages determine an oscillatory path that is distinct for each mass to charge ratio. The detector lies off the axis of the original beam, and variation of the electronic parameters can direct any wished-for mass, alone, into the detector. These detectors are the most sensitive, the most stable, and among the cheapest. However, they have limited mass range and only moderate resolution.

DETECTOR: The original detectors were simply photographic plates, with the charged fragments acting upon the silver emulsion exactly in the same way as would a beta ray or a photon.

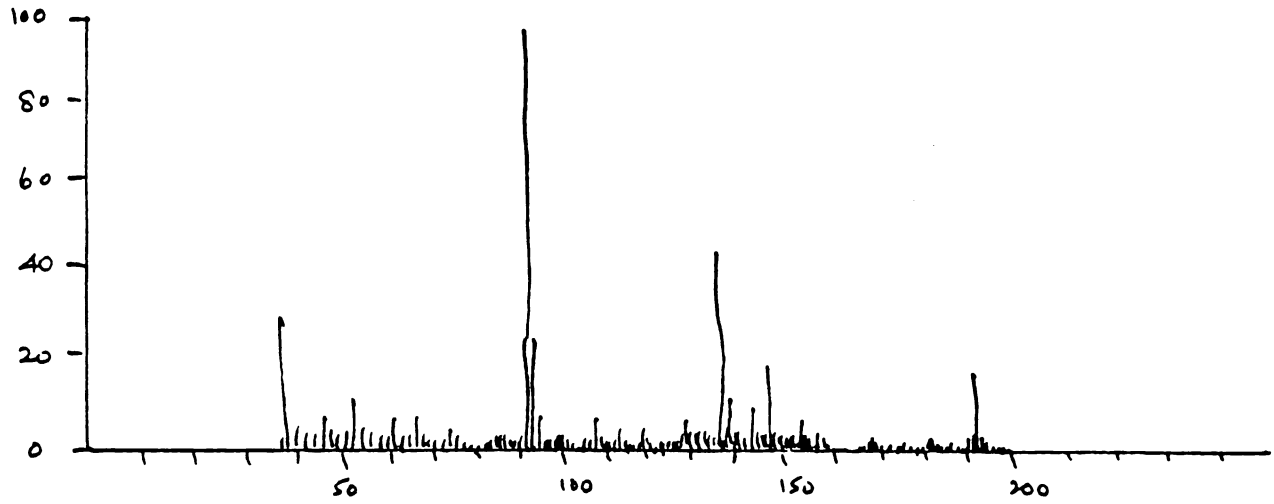
The development of the various multiplier tubes has made their use standard, but they are stationary and the fragments to be assayed must be directed to them. The electron analogy to the photomultiplier tube is the electron multiplier tube, and rather than using a phosphor, a metal plate releases electrons when hit by a charged fragment. The response time of these devices is very high (in the nanosecond range) and so actual pulses are counted, rather than the measurement of some generated current. Thus they are intrinsically linear in response.

THE USE OF THE MASS SPECTROGRAPH IN ANALYSIS

The largest inventory of mass spectral information available to the analytical chemist is associated with the electron impact (EI) mode of analysis, and it is this process which is gaining wide use in connection with GC.

The EI fragmentation spectrum is in fact a fingerprint of the compound being analyzed. With attention paid to the control of the several variables that can modify the spectrum, the identity of an EI spectrum of an unknown sample, with one from a standard exemplar, gives a strong argument for positive identification. The variables to be watched are: voltage of the ionizing electrons, the voltage parameters for the lenses and accelerators, the actual procedure chosen for mass analysis, and the vehicle used for the tuning of the quantitative responses of the mass analyzer. And when this spectral information is successfully coupled to a correct retention time on the gas chromatographic half of the analysis, the evidence for identification becomes exceptionally strong. An added strength is the vanishingly small quantity of material needed for the analysis.

Presented below is a hypothetical EI spectrum for some suspected drug, or some effluent from a GC separation.



Direct your attention to a number of things.

First, there are quite a large number of peaks, indicating extensive variation in fragmentation. This is characteristic for an EI spectrum; a CI spectrum usually has parent peaks and the expected satellites, but little else on either side of the parent peak.

A second quality shown is the fact that the Y-axis is from 0 to 100. With EI spectra, it is a convention to present all peak heights normalized with respect to the strongest peak in the spectrum. It becomes 100%, and all others are reported proportionally to it. So here is the peak at a mass of about 91 that is the strongest peak in the spectrum. Look at the peak at about 193. This is the parent peak, or so one might guess since it appears to be the highest mass peak in the spectrum. Below is a more amplified and expanded presentation of that part of the spectrum.

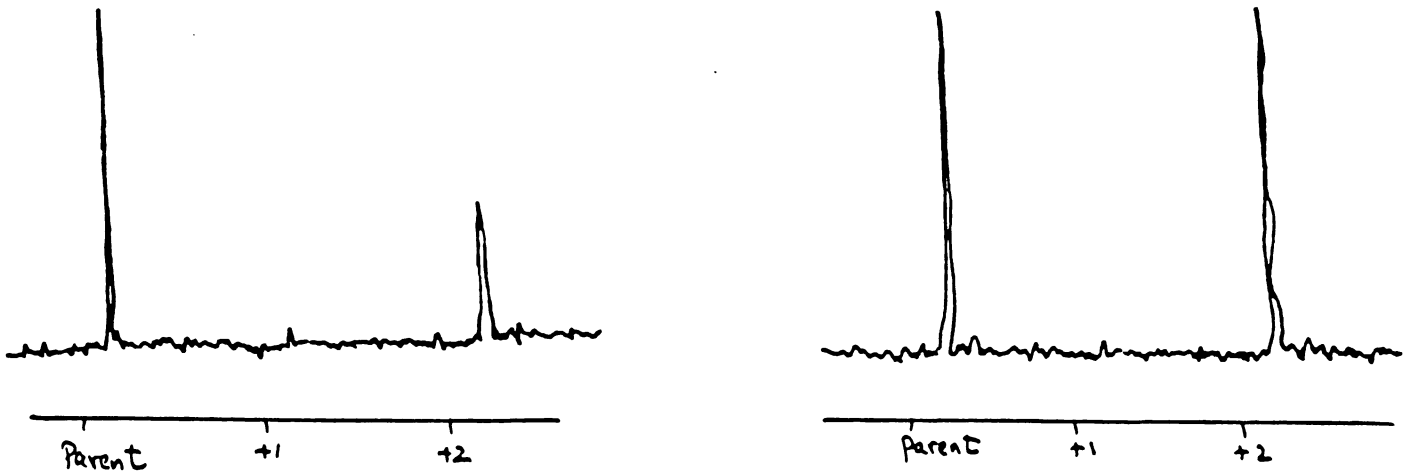
Let us assume that 193 is the nominal molecular weight of the parent drug we are analyzing. What are the actual things that are banging away on the electron multiplier tube? Recall the opening paragraphs of Lecture #15. You are looking at this material molecule by molecule, and statistics are not to be applied. That means that if you could grab an electron deficient and thus positively charged molecule just before it hit the detector, you would find that every carbon atom in it was uniquely mass 12, and that every hydrogen atom in it was uniquely mass about-one. And if it had oxygen, the atom in that grabbed molecule had to be mass 16, and if it had nitrogen, that N atom had to be the isotope 14. Had there been a single deuterium, or ^{13}C, or ^{15}N in the grabbed molecule, it would have appeared at mass "about-194."

So, look at mass "about-194." There, slightly to the right of the calibration mark, there is a small but real peak. Assuming that the compound, the drug, is pure, and assuming that everything is calibrated OK, the 194 peak must be the parent molecule with one excess mass. So there was one of the hydrogens that was deuterium, or (not and) one of the carbons was ^{13}C, etc., etc. If you had one molecule in hand that had BOTH a ^{2}H AND a ^{13}C, it would emerge at a mass of about 195, and indeed there is a small wiggle there at that location in the above picture.

NITROGEN, THE ODD-MASSED PARENT PEAK, AND DOUBLE PARENT PEAKS

An easily overlooked point of information is the fact that the parent peak, the 193 peak, is an odd number. All hydrocarbons have an even number of hydrogens, and since carbon atomic weight is nominally an even number (12), all hydrocarbons have an even molecular weight. Adding oxygen to the recipe (a divalent O atom with an even weight) does not change the picture. Nor does sulfur at 32. And the halides all have an odd atomic weight, but they are like hydrogen, monovalent, and again the picture is not changed. The only atom that violates this symmetry (odd valency, odd mass or even valency even mass) is the atom nitrogen. So the having of an odd parent molecular weight demands that there be one (or three or five) nitrogen atoms present. This is the only atom that can produce an odd parent peak.

So, a drug with an odd mass molecular weight parent ion contains nitrogen. Another point of information can be gathered from the parent peak concerning the halides chlorine and bromine. Chlorine, as she is found in nature, is approximately a 3:1 mixture of ^{35}Cl and ^{37}Cl, so if the drug contains an atom of chlorine, the parent peak would have appeared as below. There would have been a parent+2 peak of fully a third the size. And, if the drug contained an atom of bromine, where the ratio of the natural isotopes is almost exactly 50:50 between ^{79}Br and ^{81}Br the parent+2 peak would have been essentially the same size as the parent peak itself. The presence of

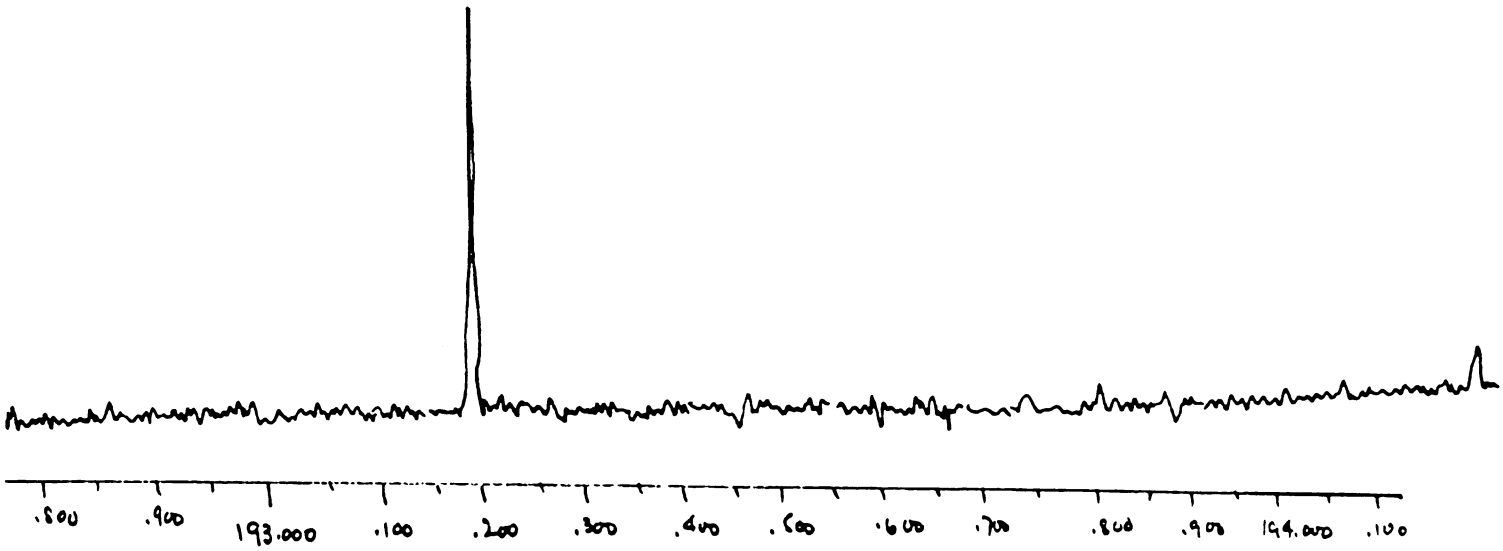


an atom of fluorine (a single isotope at mass 19) or of iodine (a single isotope at mass 127) produce normal, even-massed, parent peaks. But, if there is any chlorine or any bromine in the structure of the parent drug, it will be obvious in the mass spectrum. Multiple halides (chlorine or bromine) produce complex parent peaks, but onl;y nitrogen produces an odd-massed parent peak. And do remember, that two nitrogens (not uncommon in the drug area) will bring the mass of the parent peak back again to an even number.

So our drug has a molecular weight of 193, and probably contains a nitrogen atom (or three nitrogen atoms) and no middle halides.

DOUBLE FOCUS (OR HIGH RESOLUTION) MASS SPECTROGRAPHY

There is a procedure that is now easily available, that can focus once again on the parent peak, and reveal yet additional detail. This is called high-resolution or double focus mass spectrography. If one takes the flow of ionized molecules that is being assayed as the parent peak (often modest in amount in EI but probably a major resource in CI) and expands the entire mass spread of 193-194 AMU over the entire sheet of paper, one can get a picture as is shown below. (AMU by the way stands for atomic mass unit and represents the nominal integer near the isotope combination being looked at).

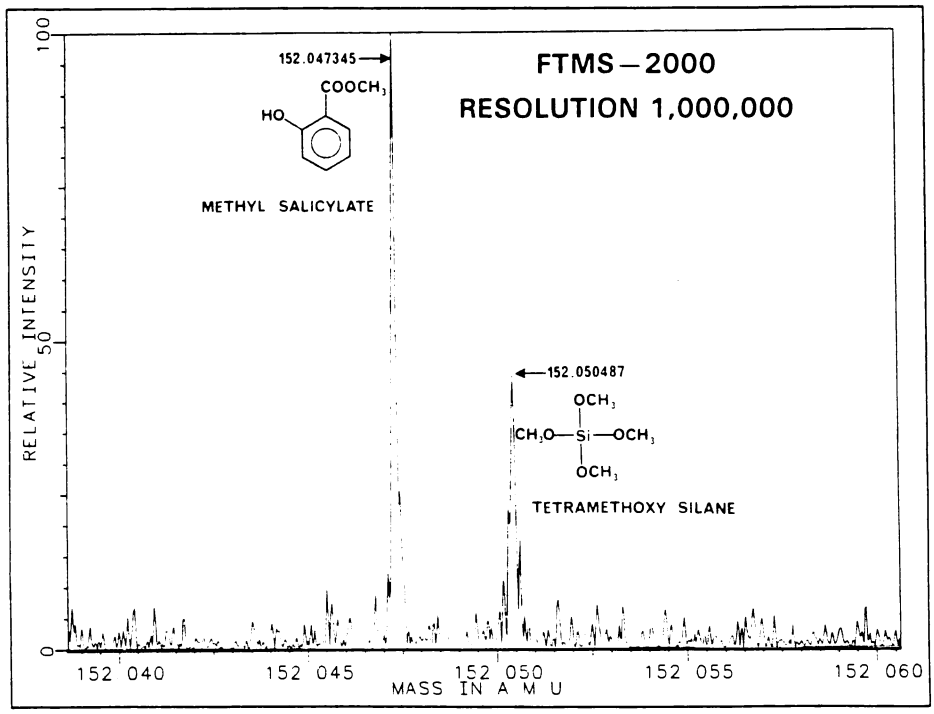


We know that the molecular weight is 193, that there is (probably) one nitrogen, and that there are no freak atoms. So, with the help of our logical friends, it is reasonable that the empirical formula is $C_{11}H_{15}NO_2$, $C_{12}H_{19}NO$ or $C_{13}H_{23}N$. Let's look at the exact masses of these molecules:

C_{11}	132.	C_{12}	144.	C_{13}	156.
H_{15}	15.1173780	H_{19}	19.1486788	H_{23}	23.1799796
N	14.0030744	N	14.0030744	N	14.0030744
O_2	31.9898298	O	15.9949149		
	193.1102822		193.1466681		193.1830540

Notice that the masses differ from one another in the second place. And with the double focus technique, a good number is obtainable in the fourth place, with a

reasonable guess in the fifth place. Hence, empirical formulae can be obtained with good confidence, simply from the exact mass of the parent peak, when read to one part in a million. To emphasize the extraordinary resolution I have attached an actual spectrum of a $C_8H_8O_3$ compound and a $C_4H_{12}O_4Si$ compound. With the estimation that the X-axis has about one inch between .005 AMU separations, the distance between mass 152 and 153 would be almost 17 feet.



FTMS ultra-high resolution studies. *The molecular ions of the two compounds in this spectrum differ by only 3 millimass units. Resolution of 1,000,000 allows us to measure the mass of one ion relative to the other with an accuracy of ten parts in 10⁷ (10 ppb)!*

GCMS: The usual term for the mass spectrometer when used as a detector for the GC, is the MSD, or mass selective detector. It is a case of having a $40,000 tail wagging a $10,000 dog. But as a detector, it has the practical signal-to-noise ratio of a N,P detector, and is thus perhaps 2 or 3 orders of magnitude more effective than a FID.

Total ion current: The use of a mass spectrograph in its most sensitive mode, would be the recording of all ionic species, regardless of molecular weight, as signals from the GC. A typical selective ion detector can record one or two thousand AMU's per second, so for practical purposes, there is a record in memory of some three complete MS scans per second. So, if a peak appears on a capillary column over the course of a second, there are some three spectra on record of the material coming off the column. A typical GC run will involve hundreds of spectra. If all of the electro-multiplier tube output signals are summed together, the sensitivity of this procedure is in the low nano-grams of material at the detector. Remember, with modern

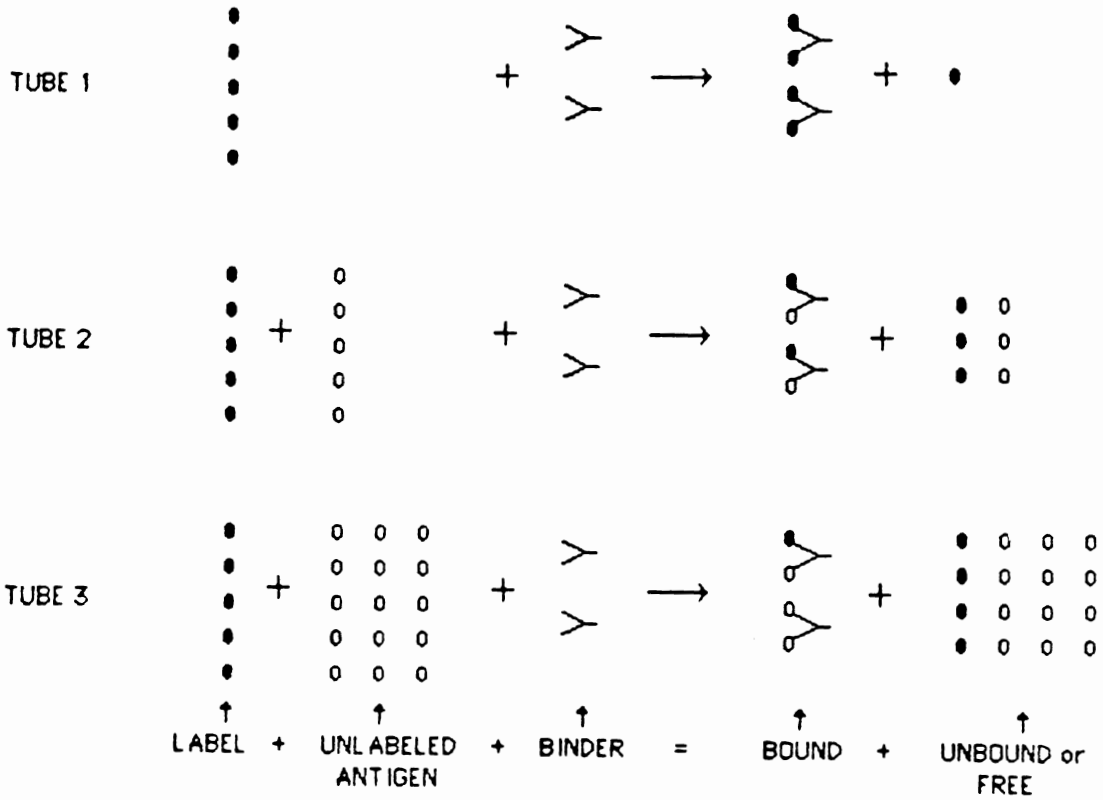
computer memories and computer speeds, every single event is recorded for future restructuring. There are several ways of reviewing this wealth of material.

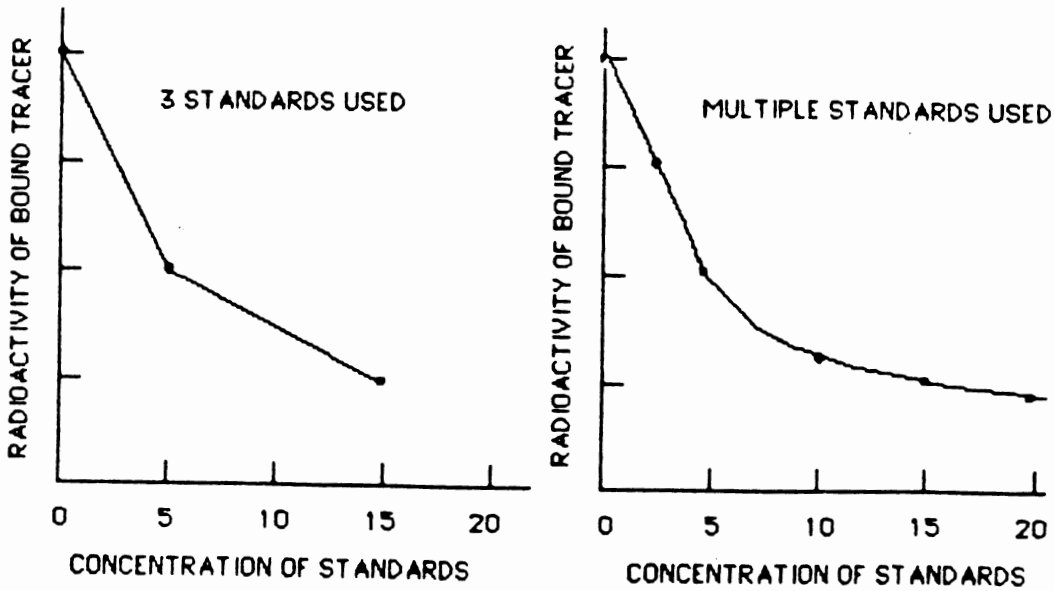
Mass spectra of individual GC peaks: In retrospect, one may recall a given GC peak, and bring up one or several mass spectra that were recorded at the moment of its emergence from the GC column. As mentioned above, the retention time coupled with the EI cracking pattern, can create an effective argument for the assignment of structure to an unknown.

Extracted ion monitoring: Rather than bringing out an individual mass spectrum, one may request a redrawing of the entire GC chromatograph, searching for a single AMU output. Thus one has a presentation of a GC run looking for a particular cracking pattern fragment which might be most characteristic of the compound being sought.

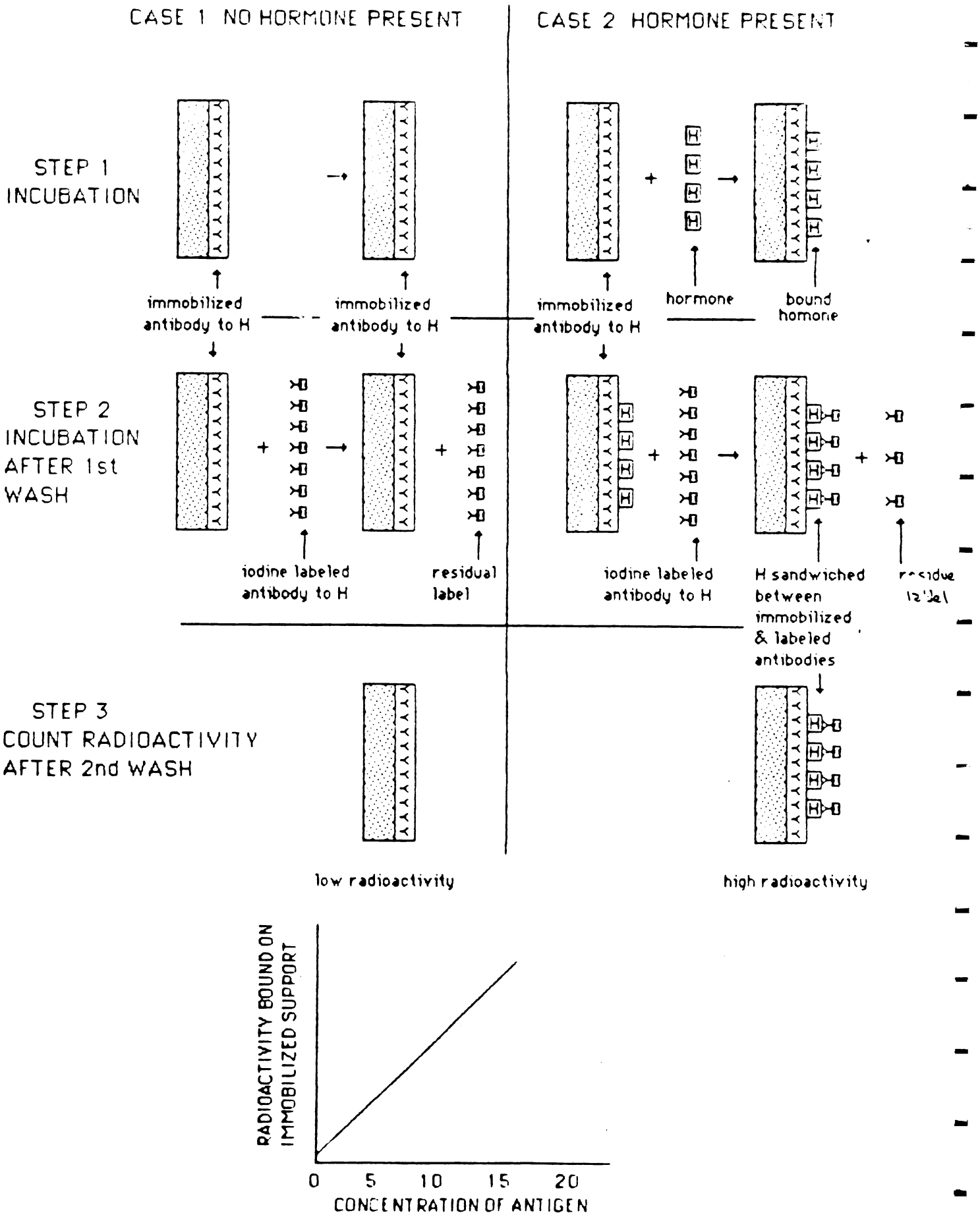
Selected ion monitoring: A great leap upwards in sensitivity may be had by letting the GCMS instrument look only for a single fragment, rather than scanning the entire spectrum routinely. Since there is some 1000 or so AMU's reachable per second, looking at a single mass location rather than the entire spectrum will allow a 1000-fold increase in sensitivity, placing the limits of detection down in the low picogram levels. And it is here that the GCMS can be an unequaled tool for toxicological examination.

PRINCIPLES OF RADIOIMMUNOASSAYS





IMMUNORADIOMETRIC ASSAYS (IRMA)



RADIOIMMUNOASSAY

1. SEPARATION OF BOUND FROM UNBOUND

a. PRECIPITATING METHODS. Use of ammonium sulfate, sodium sulfate or some other precipitating agent for separation. There is a requirement that the precipitating agent does not precipitate the unbound or radioactive analyte. Though the methods are generally quite good, they are not in common use in the clinical laboratory.

b. COATED CHARCOAL METHODS. Dextrans coated on charcoal act as dialysing membranes and prevent the antibody bound analyte from being absorbed onto the charcoal. It is difficult to dispense charcoal for large runs. This method has become increasingly rare.

c. ABSORPTION METHODS. Talc, silica, bentonite, resins, staphylococcal protein A, and other materials have been used to separate the bound from unbound. Not in use in the clinical laboratory.

d. DOUBLE ANTIBODY METHOD. Use of a rabbit antibody to the antigen; then precipitate the first antibody with a second antibody (such as goat anti-rabbitt gamma globulin). Has become increasingly popular and now quite common since the discovery of the use of polyethylene glycol as an accelerant of the precipitation step with the second antibody.

e. IMMOBILIZATION OF THE ANTIBODY: USE OF INSOLUBLE ANTIBODY SUPPORTS. Antibody can be coated on plastic tubes or beads, or attached covalently to cellulose, glass beads, or other types of insoluble materials. Use of these materials is becoming increasingly more common. Reagents tend to be a little more expensive because of manufacturing costs. The use of insoluble supports is a requirement for most of the IRMA methods.

f. OTHER METHODS. Chromatoelectrophoresis (the original method of Berson and Yalow). Gel filtration. Magnetic methods.

2. IODINATION

a. CHLORAMINE T METHOD of HUNTER AND GREENWOOD. Radioactive I-125 sodium iodide is oxidized to I_2 with the mild oxidizing agent Chloramine T. The I_2 then reacts with a tyrosine in the protein. After a short time, the oxidizing reagent is then reduced with a mild reducing agent, metabisulfite. The products are then separated on a gel filtration column with the higher molecular weight materials emerging first. At times one uses other forms of chromatography for separation.

b. ENZYMATIC IODINATION. Use of lactoperoxidase methods and H_2O_2.

c. ATTACHMENT OF RADIOACTIVE DERIVATIVES TO THE ANALYTE. The Hunter Bolton reagent. Cost factors.

d. IODINATION OF SMALL MOLECULAR WEIGHT MATERIALS. Use of tyrosine methyl ester, tyramine, or similar compounds covalently attached to small molecular weight materials. The tyrosine derivitives tend to be preferentially iodinated with the Hunter-Greenwood Chloramine T method. Purification generally by chromatography.

3. BINDERS

a. NATURALLY OCCURRING BINDERS. INTRINSIC FACTOR for Vitamin B12. Milk binding protein for folates. Cortisol binders from horse serum. Thyroxine Binding Globulin (TBC) for T_4 assays (Murphy-Pattee Method).

b. SPECIFIC ANTIBODIES TO PROTEINS. Generally prepared by classical methods.

c. ANTIBODIES TO SMALL MOLECULAR WEIGHT MATERIALS. Haptens (such as digoxin) covalently coupled to foreign proteins (for example, bovine albumin) act as immunizing agents. Animals will make antibodies to the foreign protein, to the hapten, and sometimes to the bridge between the hapten and the protein.

d. SPECIFICITY OF THE ASSAY. Remember, the specificity of the assay comes from the antibody ... binder. If the binder is highly specific and will not bind materials other than the analyte of interest, then we say the assay is highly specific. If the binder binds similar materials (such as similar steroids), then there is considerable crossreactivity and the assay is not particularly specific. The specicity of the assay can, however, be considerably increased by purifying the analyte. For example, some steroids are purified by chromatography before being analysed by a radioimmunoassay.

4. ASSAY CONDITIONS

a. TYPICAL CONDITIONS. Add 100 ul of standards, controls, or samples to the appropriate tubes. Add 100 ul of I-125 labeled TSH to each tube. To all tubes except the first 2 tubes containing a zero standard, add 100 ul of the rabbit anti-human TSH. Incubate for 2 hours. Add 1 ml of the second antibody (goat anti-rabbit gamma globulin) containing the precipitating accelerant, polyethylene glycol. Wait 5 minutes, then centrifuge for 20 minutes. Decant the supernatant off the precipitate and allow the tubes to drain onto an absorbant paper for a few minutes. Count for radioactive I-125 and calculate results.

5. CONSIDERATIONS

a. ENDOGENOUS BINDERS NEED BE ELIMINATED. In the Vitamin B12 and Folate assays, the naturally occurring binders are destroyed by boiling the sample. In some assays, the analyte is extracted from the binder with organic solvents. In the Thyroxine assay (T4), a displacing agent is added to the sample to displace T4 binding to thyroxine binding globulin (8-anilino-naphthalene sulfonate or ANS).

b. RIA AND IRMA ASSAYS. In RIA, antibody is the limiting reagent. In IRMA assays, the antibody and labeled antibody are in excess.

c. RIA is a competitive assay. IRMA assays are not competitive assays.

d. RIA and IRMA assays are considered NON-HOMOGENEOUS assays. There is a requirement for the separation of bound from unbound before one can count the radioactivity. In HOMOGENEOUS assays, the signals are modified or attenuated by the binding of the antibody to the labeled antigen (such in fluorescent polarization methods for therapeutic drug monitoring, the TDX system of Abbott, or the EMIT methods developed by Syva).

6. TERMINOLOGY and DEFINITIONS.

a. Tc. TOTAL COUNT RATE. This is the total radioactive count rate for the radioactive material added to each tube.

a. NSB or NON SPECIFIC BINDING. This is the non-antibody bound radioactive count rate that is absorbed onto counting tubes or beads, is contained in precipitates, or is occluded in a precipitate. The NON SPECIFIC BINDING count is subtracted from any count that is obtained after binding by the specific antibody.

c. B'. The raw count rate of a standard, control or sample not corrected for NSB.

d. Bo. Corrected count for the 0 standard. The raw count rate at the zero standard (B') is corrected by subtracting the NSB count. The resulting count rate, Bo, is then attributed to the specific binding by the antibody.

d. B. Corrected count for other standards, controls or samples; all corrected by subtracting the NSB count.

e. Bo/Tc. The NSB corrected count for the zero standard divided by the total count for each tube to give the fraction of radioactivity bound by the antibody without the interference of other competitive components. Usually expressed as a percentage; Bo x 100/Tc.

f. B/Bo. Ratio of the count rates for standards, controls or samples divided by Bo. All count rates are corrected by subtracting the NSB count rate. This ratio varies from 1 to close to zero (or from 100% to a very low percentage) and is very commonly used in most RIA calculations.

g. NSB/Tc. Ratio of the non specific binding count to the total count added to each tube. Usually used as a quality control figure.

7. CALCULATION METHODS.

a. COUNT RATE against STANDARDS.

b. B/Tc versus STANDARDS.

c. B/Bo versus STANDARDS.

d. B/Bo versus LOG STANDARDS.

e. LOGIT B/Bo versus LOG STANDARDS.

f. POINT TO POINT CURVES.

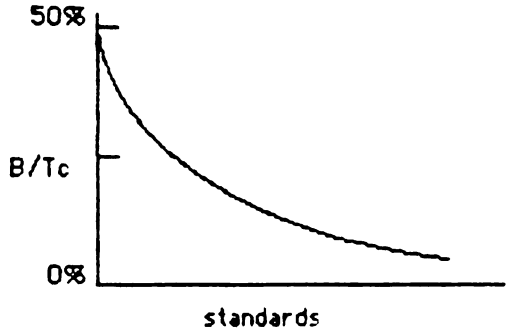
g. POLYNOMIAL FITS.

See next page.

B. GRAPHICAL PRESENTATIONS

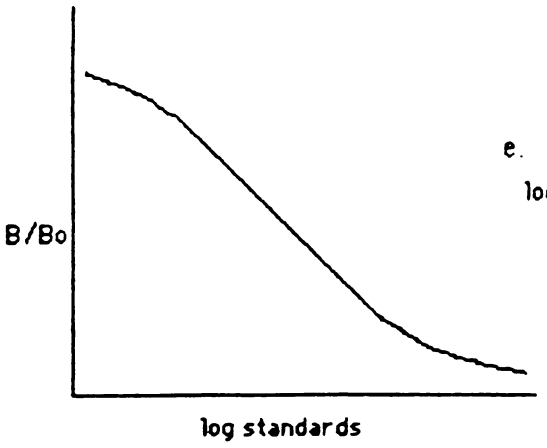
a.

b.

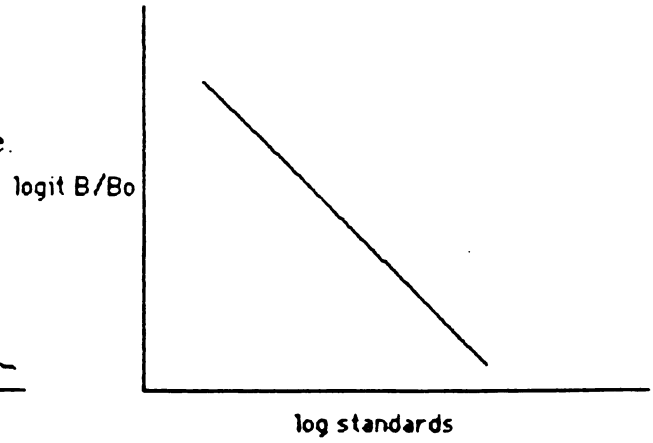


c.

d.



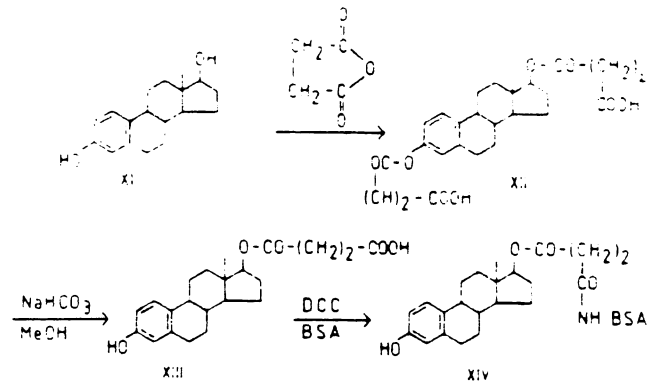
e.



$$\text{logit } B/Bo = A - B \times \log C$$

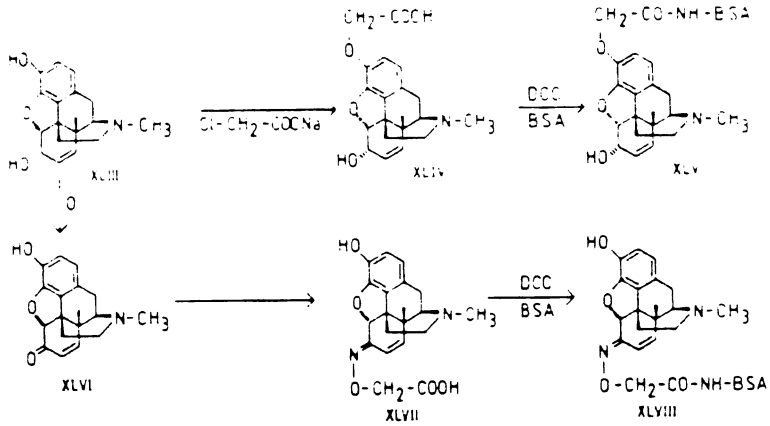
$$\text{logit } B/Bo = \log_e (B/Bo)/(1 - B/Bo)$$

PREPARATION OF SOME DERIVATIVES FOR RIA



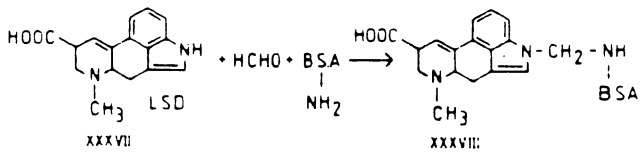
17β-Succinyl Estradiol-Bovine Serum Albumin.

Estradiol(XI) treated with succinic anhydride in pyridine yields the 3,17β-disuccinyl estradiol (XII). On mild alkaline conditions, this compound is hydrolysed to the 17β-monosuccinate of estradiol(XIII). Using the carbodiimide reaction and BSA, this monosuccinate is coupled to Bovine Serum Albumin to give (XIV).



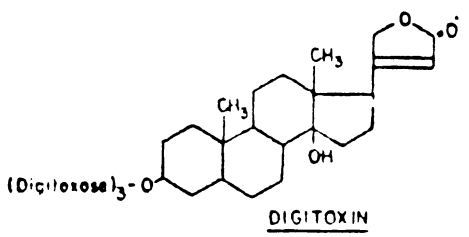
Morphine-Bovine Serum Albumin Conjugate

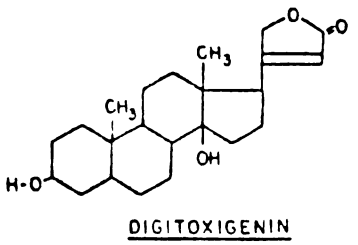
Morphine (XLIII) yields the 3-carboxymethyl ether (XLIV). With the carbodiimide reagent and BSA, this compound is conjugated to BSA(XLV). Morphine (XLIII) with an oxidizing agent gives morphone (XLVI). Treatment of this compound with carboxymethoxylamine.HCl yields the 6-carboxymethyloxime of morphone (XLVII). This compound with BSA and a carbodiimide reagent gives the BSA conjugate (XLVIII).



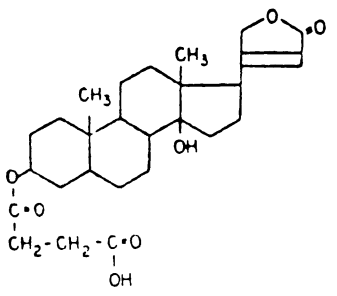
Lysergic Acid-BSA Conjugate

A Mannich reaction couples BSA to Lysergic Acid. The indole nitrogen of LSD is linked to the BSA through a methylene bridge.



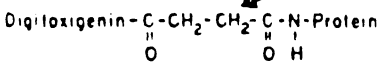


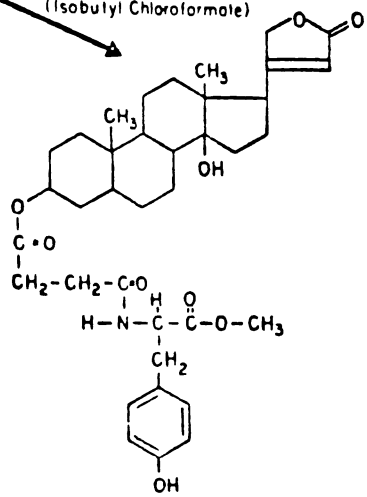
Succinic Anhydride



Protein-NH_2 (Carbodiimide)

Tyrosine Methyl Ester (Isobutyl Chloroformate)





3-O-Succinyl Digitoxigenin Tyrosine Methyl Ester

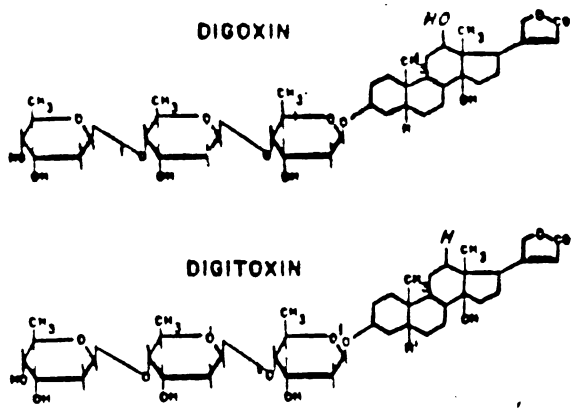


Figure 1. Structural Formulas of Digoxin and Digitoxin. The steroidal portion is on the right, and the digitoxose sugars from the glycosidic site on the left.

From Measurement of Digitoxin in Human Serum by Radioimmunoassay
G. Oliver, Jr., B.M. Parker, D.L. Brasfield, & C.W. Parker
J. Clinical Investigation 47 1035 (1968)

TUBE #	TUBE CONTENTS	COUNTS/MIN CPM	CPM AVERAGE		
1	Total Count	20,050			
2	"	19,950			
	Standards ng/ml				
3	0	16,300			
4	0	15,700			
5	0.5	11500			
6	0.5	11300			
7	1	7980			
8	1	8020			
9	2	5800			
10	2	5700			
11	3	4100			
12	3	3900			
13	4	2900			
14	4	2800			
15	6	1700			
16	6	2300			
	CONTROLS			CONC.	CONTROLS OK?
17	Control 1	8200			
18	Control 1	8400			
19	Control 2	5500			
20	Control 2	5600			
	SAMPLES				Speculations on the patients.
21	Steven H.	7000			
22	Steven H.	7200			
23	Gloria K.	6000			
24	Gloria K.	6300			
25	David P.	10100			
26	David P.	9900			
27	Mary L.	4700			
28	Mary L.	4900			
29	Nancy F.	15200			
30	Nancy F.	14900			

Normal therapeutic levels: 0.7 - 1.7 ng/ml.

Toxic range: greater than 1.7 ng/ml.

lab m'p2 10/20/85

WORKSHEET FOR A DIGOXIN ASSAY

DIGOXIN ASSAYS

The name 'digitalis' covers those compounds that may be extracted or derived from certain plant materials (particularly the foxglove) and which exert certain beneficial effects on the heart. Digoxin is a derived member of the digitalis group obtained from 'Digitalis lanata' and is very commonly used for the treatment of conjestive heart failure.

There is a very narrow therapeutic range for digoxin; if the patient fails to take the drug, or if adverse physiological conditions such as uremia exists which will lead to toxic levels of the drug, then the patient might well be in serious jeopardy. Thus, digoxin assays are quite commonly utilized by physicians for patients that may have doubtful physiological status.

A TYPICAL DIGOXIN ASSAY

There are numerous types of digoxin assays commercially available. Most of the advertised benefits of these commercial materials emphasize accuracy and rapid turnaround time for results. The assay here is a commonly used double antibody assay. The accelerator for the precipitation step (the second antibody step) is a low concentration of polyethylene glycol. The assay is usually done in duplicate.

100 microliters standards, controls or samples.
100 microliters of an iodinated derivative of digoxin.
500 microliters of a rabbit antibody to digoxin.

Incubate for 1 hour at 37^0 C. Then add the second antibody (a goat anti-rabbit gamma globulin). Incubate a further 10 minutes. Centrifuge for 20 minutes, then decant the supernatant and carefully drain the tubes. Count the radioactivity in the precipitate for 1 minute.

Draw a standard curve by plotting the average of the count rate of the duplicate standards against the value of the standards on linear linear paper.

Read off the values for the controls and for the samples.

Knowing the therapeutic range (0.7 - 1.7 ng/ml) for digoxin, speculate on the status of the patient.

HOMOGENEOUS IMMUNOASSAYS:

The radioimmune assays discussed during the last hour are all intrinsically heterogeneous. As a most simplified model, there is an unknown amount of drug (or analyte) in solution in, say, urine. A known amount of distinguishable, "seeable" material is added, a material that will react similarly to the analyte, to associate with the antibody. Then an insufficient amount of antibody is introduced, which competes for the native analyte and for the introduced "seeable" material. When an equilibrium has been established (analyte and "seeable" material competing for the inadequate amount of antibody) a separation of some sort is made (thus heterogeneous) of bound material from unbound material. The proportion of "seeable" stuff in either of these two separated fractions constitutes the assay.

The process is identical for a homogeneous assay, except that the separation step is not needed. A form of "seeable" material is used, so that the extent of its having been bound to the antibody can be determined by a change in the quality or the availability of the thing seen.

EMIT:

The homogeneous technique known as EMIT uses an enzyme as the "seeable" aspect of the analysis. As an illustration, consider the first commercially successful example of the procedure. This was an analysis for morphine (the analyte) using the enzyme lysozyme (an enzyme which digests and thus solubilizes bacterial cell walls) as the "seeable" component.

The antibody component in the analysis is the same as is found with heterogeneous assays – an antibody is generated to an antigen containing morphine, and this antibody must be cross-reactive to morphine itself.

The antigen side of the assay is quite different than with radioimmune analyses. The enzyme lysozyme has, on its outer surface and near its active site, an amino group (actually the terminal amino group of the amino acid #47, lysine). If the intact enzyme is placed in contact with suspended bacterial cell walls, there will be a predictable rate of digestion. This amino group is chemically coupled with a molecule of morphine which, although adding some steric bulk to the side of the enzyme near the active site, still does not appreciably interfere with the enzyme's activity. The morphine-modified enzyme, when placed in contact with bacterial wall material, will digest away as if the amino group was completely free and unencumbered. But if this morphine-modified enzyme is exposed to an antibody to morphine, then the entire face of the enzyme, including the active site of action, is obscured, and there is no longer an enzymatic activity.

And one has a classic antigen-antibody assay. A urine sample, with an

unknown amount of morphine present, is treated with a known amount of morphine-modified enzyme. Both species are able to bind to the anti-morphine antibody. And when an insufficient amount of anti-morphine antibody is then introduced into the assay, the two species will compete for it, interchangeably. The more morphine present in urine, the more it will bind to the antibody at the cost of the morphine-modified enzyme, and so the more of the enzyme will be free and show activity. Once this entire homogeneous mass is assembled (morphine, morphine-modified enzyme, antibody) a suspension of bacterial wall material is added, and the entire thing placed in a spectrophotometer. The faster the solution clears (digestion of the cell walls) the more there must have been free morphine in the original solution, for it has preferentially (statistically) usurped the limited amount of antibody. And this speed of loss of optical density is a non-linear measure of the absolute quantity of morphine present. Standard curves are absolutely necessary, with known amounts of morphine in drug-free urine establishing a response curve.

More recently, other enzymes such as glucose-6-phosphate dehydrogenase, malate dehydrogenase and beta-galactosidase, have served as labels. The free enzyme activity can be determined spectrophotometrically by the associated NAD-NADH or NADP-NADPH couples associated with it.

Many variations of this theme have been explored and are found in analysis. One can find the use of coupled enzyme reactions (where one component is bound to a solid phase, the other to the actual antibody), the enzyme cofactors can be the covalently bound material, and even materials that are capable of modifying enzyme activity (as inhibitors) may be the labels.

FRAT:

An entirely different approach to the homogeneous assay is seen in the use of electron spin resonance assays, but the general principle is the same.

Again, an antibody is generated to the analyte and only the "seeable" antigenic factor is modified. Here, a derivative of morphine is prepared that carries as part of its structure a stable free radical. A substituted morphine that can be coupled onto a BSA (bovine serum albumin) to be antigenic, can through the same linkage be bound onto an amine which is attached to a hindered N-oxide such as 2,2,5,5-tetramethylpyrollidine-N-oxide. This free radical has a electron spin resonance that is distinct in the EPR (electron paramagnetic resonance) spectrophotometer. The intensity of the spin resonance, however, depends upon the mass of the molecule that carries the spin. Hence, if the morphine-radical combination is unencumbered, it will have much tumbling, it will have a strong resonance. And the stage is set again for a competitive grab for antibody. There is an unknown amount of morphine in the urine, a known amount of morphine-to-radical "seeable" material is added, and then an insufficient amount of antibody. The competition is on. With larger amounts of morphine as analyte present, the more it will succeed in consuming the antibody and the larger the amount of radical-bound morphine will be in free solution. And the larger the spin resonance signal. Again, there is a correlation (albeit non- linear) between the signal

and the morphine present in urine.

A very similar procedure has recently become very popular; florescence polarization. In the very simplest terms it is an exact analogy to the FRAT system just described. In short, the "seeable" antigen is a morphine molecule (to stay parallel to the above examples) that has been covalently coupled to a fluorescing chromophore. In a polarized environment with a spectrophotometric polarimeter, the loss of polarization will be a function of random movement, tumbling, which in turn will be a measure of the mass (inertia) of the fluorescing molecule. The parallel should be apparent. The larger the amount of analyte (the free morphine in the urine), the more it commands the antibody. And the less of the antibody is available for the fluorescing morphine antigen "seeable" factor. Hence, the lower its average molecular weight, and the faster the polarization is lost. Again, a correlation between speed of depolarization and analyte present is the assay.

HEMAGGLUTINATION INHIBITION:

An immunoassay that is rarely seen in forensic work, but has had wide use in the clinical laboratory, is hemagglutination inhibition. The attached handout illustrates this technique, again, using morphine as the demonstration analyte.

As with most other assays, morphine is covalently bound to a molecule large enough to be antigenic, but this now serves two roles. First, it is the stimulant for the formation of antibodies in some animal. But it also is used as an absorbed material on the surface of sensitized erythrocytes (RBCs). These cells, when suspended in a liquid medium, will normally settle out to form a pellet, a dark spot at the bottom of the tube. The assay is set up to a predetermined break point of analyte sensitivity, and will give results only as being greater than or less than, that break point. In other words, a drug is not measured quantitatively, but simply is it there or not.

A long row of tubes containing drug-free urine, are spiked with varying amounts of analyte, and a constant but limited amount of antibody is introduced. There will be a point at which the drug present will exceed the capacity of the antibody present. This is towards the left side of the accompanying handout. A fixed quantity of sensitized and antigen-coated RBCs are then introduced, and in those tubes where there has been antibody in excess (small amounts of drug initially present) the RBC becomes associated with the antibody and becomes agglutinated (forms a matrix that keeps the cells from settling out to the bottom of the tube and forming a pellet). And where the antibody has already been consumed, the RBCs are present in excess, and they form a normal pellet.

Complex? Yes. Temperamental? Extremely. A capricious assay that can be no end of trouble. But when it works, it can process large volumes of unknowns in minutes very cheaply. Remember, a pellet is positive!

CROSS-REACTIVITY:

The presence in urine of metabolites of a drug, or of different chemicals with somewhat similar structures, represents a complication for any immunoassay that must be carefully thought tl.rough. These factors can be curses or they can be blessings.

Cross-reactivity is the observation that all things serve to some degree as antigens to a given antibody. In the above examples we assumed that the drug being tested for, the analyte, was equal in attraction to the analyte-to-enzyme, or analyte-to-radical competitor. But they are not exactly the same molecules, and so do not have exactly the same binding forces. And there can be many, many closely similar molecules that could be present and which might serve as unexpected competitors for the antibody.

Cross-reactivity as a blessing. This is the case as a rule when one is looking for classes of drugs, or for the presence of metabolites that might imply the use of a drug. Let us assume that one is working with an antibody to methamphetamine. In any initial evaluation of an immunoassay, one tries all the closely related drugs that might possibly be present in urine, to see how much of a response they might give to the antibody. If methamphetamine gives a response of "100%", it may be that amphetamine gives a 60% response, N-ethylamphetamine a response of 45%, and Sudafed a response of 15%. With this as a given, look at the virtues. Amphetamine is a metabolite of methamphetamine, and so if the question asked was, "Is there evidence of methamphetamine usage," it will actually increase the absolute sensitivity of the assay. If the question asked was, "Is there evidence for the use of amphetamines," any of these drugs that might be present in urine would give a positive response and give support to a positive answer. This can be especially valuable where the actual metabolites of a drug may not be well defined, but might well be of a structure closely related to the original drug. From the presence of structurally related metabolites, the use of the parent drug may guardedly be inferred. Any qualitative measure will demand that the assumption be made that ONLY the target drug was present, and the analysis must be couched in these terms. "How much methamphetamine was present?" will get an answer, "A response was found that was equivalent to the presence of 0.27 ug/ml of methamphetamine in the urine." Or, "The assay showed that, measured as methamphetamine, there was a total of 0.27 ug/ml present."

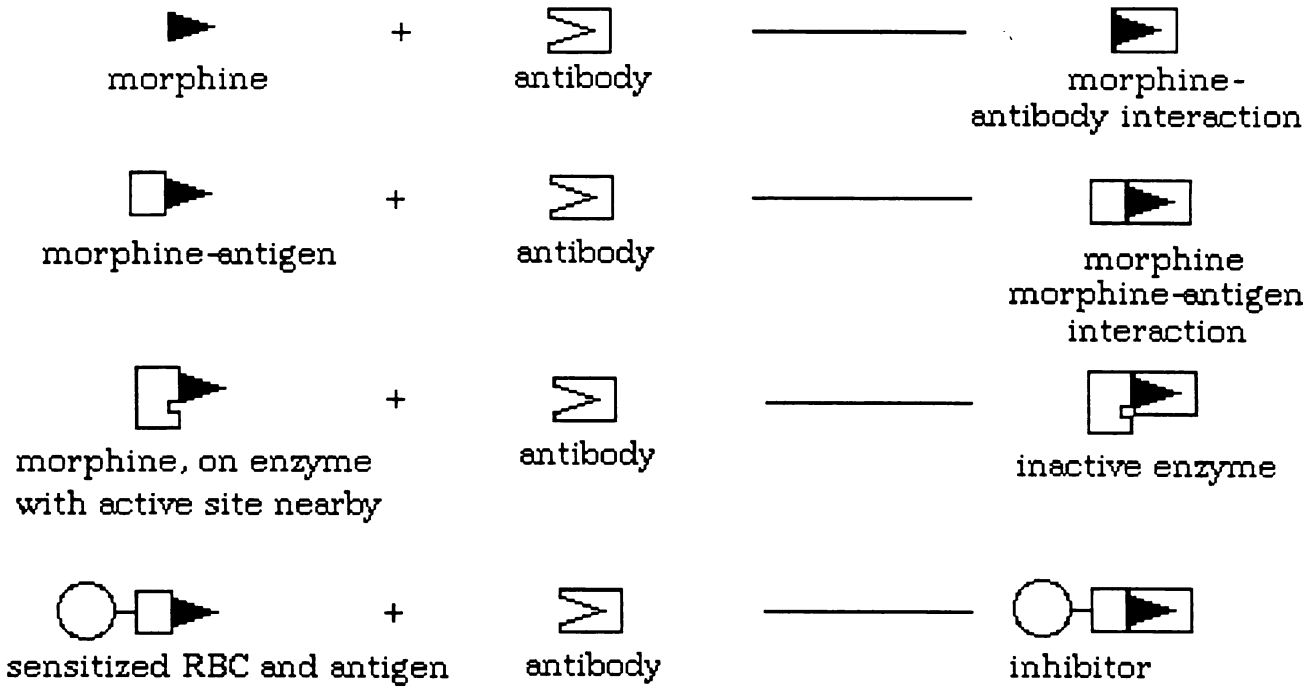
Do think over the phrasing of these answers quite carefully. They are accurate answers, and yet they are completely misleading answers to an unprepared audience. And if, in a legal examination, such an answer is followed up by a question concerning your opinion as to the loss of function a person might have experienced with a urine level of 0.27 ug/ml of methamphetamine, then the jury will have unconsciously accepted as fact that there was this amount of methamphetamine in the urine. And thus, that the person had indeed been "under the influence" or at least "exposed to" methamphetamine.

But what if the question had been, "Was there methamphetamine present in the

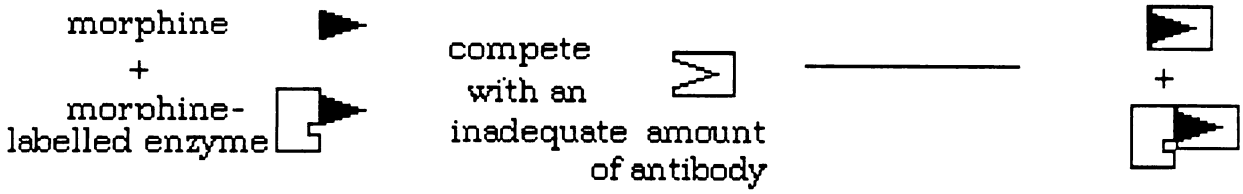
urine?" The only completely honest answer must be "I don't know." This is the curse of cross-reactivity. All you can say is that there was a certain response to the immunoassay, and methamphetamine is known to be one of the most responsive drugs in this assay. And from here on the questioning could be most embarrassing. Hundreds of drugs could be imagined, some of them well known, as giving a some cross-reactive response to the antibody in this assay. Any of them might have been the agent present giving the positive response. And not knowing the drug, the quantitative value is meaningless.

No immunoassay result, either qualitative or quantitative, can be sufficient evidence for drug usage without confirmatory analyses. And a confirmatory analysis must use some procedure other than an immunological technique. These points are becoming extremely important today, with the wave of enthusiasm sweeping everyone into the urine-test spirit, where the antigen-antibody is one of the primary tool of screening.

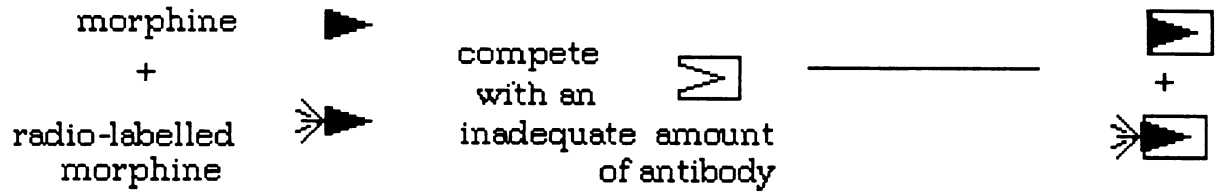
REACTIONS OF MODIFIED MORPHINE WITH ANTIBODY



THE MECHANISMS OF A HOMOGENOUS AND A HETEROGENOUS ANTIGEN - ANTBODY DRUG ANALYSIS



EMIT: A mixture of morphine and morphine-labelled active enzyme compete for a limited amount of antibody. The more morphine there is present, the more enzyme activity is observed.

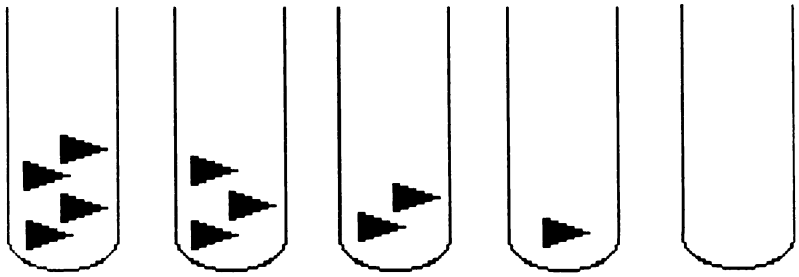


RADIOIMMUNE ASSAY (RIA): A mixture of morphine and radiolabelled morphine compete for a limited amount of antibody. The more mophine there is, the less the amount of protein-bound radioactivity. The bound must first be separated before counting.

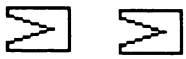
HEMAGGLUTINATION INHIBITION

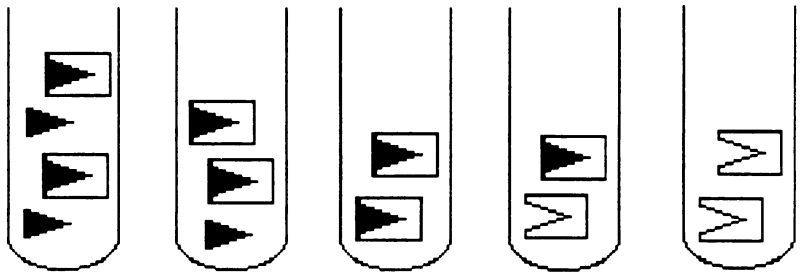
Red blood cells are sensitized with tannic acid, and then treated with antigen to form a delicate RBC that can react with the morphine antibody. When the amount of morphine in the test sample is so small that there is excess antibody left behind, then it will react with the RBC-bound antigen which allows agglutination. Remember, a pellet is positive.

To a series of tubes, there is added various amounts of morphine, bracketing the range wanted.

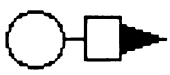


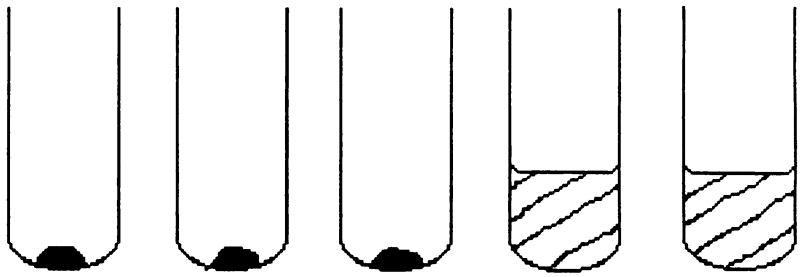
There is then added a limited amount of the morphine antibody.





And finally, there is added the sensitized antigenic



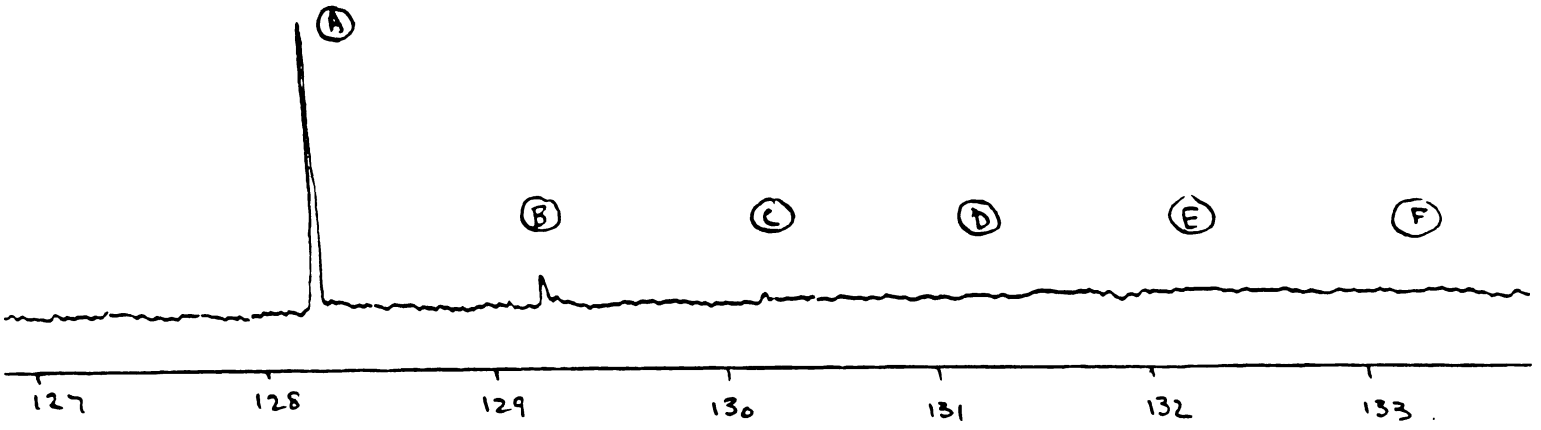


No excess antibody	No excess antibody	No excess antibody	Excess antibody	Excess antibody
Pellet	Pellet	Pellet	No pellet	No pellet

SECOND MIDTERM GIVEN IN BEHS 183 IN 1985

Midterm #2
Open Book Take-home
BEHS 183 Halloween 1985

During trick-and-treating, you are handed a capsule containing the drug Pumpkinone, a white solid with the empirical formula $C_7H_{13}NO$. You rush down to the lab and get the following CIMS:



(1) What is the exact molecular weight of the solid in the capsule?

(2) What is the exact molecular weight of the peak at A?

(3) Why is there a peak at B?

(4) What letter would be given to the highest molecular weight peak that would theoretically exist?

(5) Why is the largest peak (A) at about the mass 128, when the molecular weight of Pumpkinone is about 127?

Give one sentence definitions of:

Hapten
Ion-pair extraction
Silica Gel
Methemoglobin
Legally drunk in California
Null recorder
Aspirin
Cation exchange resin
Positron
Isotope

COMMENTS ON QUESTIONS #1,2 & 4, ON MIDTERM #2, HALLOWEEN, 1985

#1 Answers Received	Correct Answer
127 (about)	C_7 12.01115 x 7 = 84.07805
127	H_{13} 1.00797 x 13 = 13.10361
127.099717	N 14.0067 x 1 = 14.0067
127.09972	O 15.9994 x 1 = 15.9994
127.18	
127.18776	exact chemical
127.18776	atomic weight = 127.18776
127.18776	
127.18776	
127.18776	
127.1902	
127.1902	
(can't do it)	
(can't do it)	
(can't do it)	

#2 Answers Received	Correct Answer
127.09971	$^{12}C_7$ 12. x 7 = 84
127.0997169	$^{1}H_{14}$ 1.0078252 x 14 = 14.1095528
127.0997169	^{14}N 14.0030744 x 1 = 14.0030744
127.0997169	^{16}O 15.9949149 x 1 = 15.9949149
128.0997169	
128.1039342	exact physical
128.10754	(isotopic) At. Wt. = 128.1075421
128.10754	
128.1075408	
128.1075421	
128.1075421	
128.1075425	
128.18776	
128.188	
128.2	

#4 Answers Received	Correct Answer
C	In $C_7H_{14}NO$, with maximum possible
FFFF	heavy isotopes, there would be:
V	
WW	7 ^{13}C's excess mass = 7
XXXX	14 ^{2}H's = 14
Y (and several students	1 ^{15}N = 1
considered unstable isotopes)	1 ^{18}O = 2
	24

There could be a molecule with an added 24 AMU's, (and of course everything in between). Hence, 24 additional peaks are possible. The 25th letter is Y.

PRESUMPTIVE TESTS AND FIELD TESTS:

These two categories are considered as one, in that many of the techniques and reagents are shared between the two.

A presumptive test is a preliminary evaluation of a seized sample, plant, powder, thing, to come to a educated guess as to whether it might be a drug which would justify some official action. It may be a color test performed on the site of a potential arrest, and it may be a laboratory test done at leisure and out of the presence of the alleged suspect. Whatever the test, and wherever it may be performed, it is still only presumptive, and must not be used beyond the intrinsic limitations of its value.

The immunoassay presumptive test.

This is a laboratory procedure, based on the antigen-antibody techniques covered in the last two lectures. The emphasis there was that, if you know that a given drug is present (in urine, let us say) then you can measure with considerable accuracy how much of it is present. The question here is; "How much amphetamine is present?" Certainly there are many potential complications. There may be other, unsuspected drugs present as well. There may be metabolites present, in varying amounts depending on the individual, and in undetermined cross-reactive sensitivity. These can compromise the quantitative accuracy of the test. But, with the identity of the drug as a given, the amount present is potentially determinable. And from such an answer, the legal or medical questions of how much was taken, and how long ago, can been addressed.

But when the question is reversed, there is dangerous uncertainty. The question "Is there amphetamine present?" calls for a qualitative analysis, and this is the territory of the presumptive test. You cannot ever be sure from such a test alone, and you cannot with honesty even hazard a guess as to the reliability of your findings. Immunoassay shares a property with any other assay that has a background base line that is not zero: if you turn the gain up high enough you will get a reading. Many unrelated drugs may cross-react to a very small extent, but there is always some cross-reactivity if you look closely enough. Thus there has to be some arbitrary cut-off point, below which there must be the report of "negative", i.e., the drug was not present and thus the drug had not been used.

A good example of this is with nicotine. The urine level of nicotine is easily determined and has been used as a marker of tobacco use and a measure of the extent of this use with acknowledged smokers. And yet to use the finding of its presence in urine as evidence for a person being a smoker is improper in that nicotine is ubiquitous in our environment and it is in everyone's urine. And in the solvents and in the equipment and on your new polyethylene gloves and on the fresh glassware. A regular smoker may run 1000 nanograms per ml, but a person exposed to someone

else's smoke only (known as a passive smoker) will run between 5 and 10 ng/ml, and a person who is Amish and lives on a mountain top in Tibet away from everyone will still show 1 ng/ml.

A more difficult example of these problems can be seen with the urine screening for marijuana usage. The initial push for this procedure was in the military, where there are efforts to spot-check individuals for drug use. The assay was designed around the molecule THC but is acknowledged to be highly cross- reactive to many of its metabolites. But with the earliest uses of this test, the cut-off was so sensitive that it seemed a certainty that there were errors. And since the military assured everyone that there tests were 100% correct, it seemed a certainty that some innocents were hurt. These tests are only suggestive, and should be used as a justification for other, more selective, analyses.

Color tests:

Tests involving delicate equipment are not suitable in the field. The situation comes up, more in police work than in medical practice, where a suspect may have something in his possession which might be a drug, but the circumstances surrounding the police-suspect contact are not sufficiently incriminating to justify an arrest. There are several kits now available that can be carried to a possible crime scene or suspicious encounter which can give an alerting color or sign for the presence of a controlled drug. The same uncertainties are here as had been mentioned above, but the strengths are real: If the presumptive test is negative, there is a good argument for not detaining (arresting) the suspect, and if the test is positive such an arrest can be argued as having been for adequate cause. Since the testing agent is known but the drug is not, I will classify these by test agent. Always remember, that there may be false positives (some unexpected drug given the test), that these tests are aimed towards actual drug samples, not suspect urines, that dark colors can cover up light colors, and that most complex scheme trees (to finely differentiate between slightly different things) are failures.

Duquenois test. Probably the best known and one of the most studied tests for marijuana. There are many variations, but the basic chemistry involves an alcoholic solution of vanillin and acetaldehyde acidified with HCl. The end color produced is violet, and there are very few plant products which are known to give a false positive. A modification (Levine) is often added, which is the extraction of this color, once formed, into chloroform. More of the marijuana story will be covered in lecture 24.

Marquis test. Three or four drops of sulfuric acid (concentrated) is added to a suspect sample. With most abuse drugs, there is no color developed at this stage, but the generation of an immediate black color is characteristic of the generation of formaldehyde, and may indicate drugs such as MDA, MMDA or MDMA. And the generation of a blue-green color is characteristic of piperidyl esters such as the JB compounds. The Marquis test is completed by the addition of a drop of 37% formaldehyde, and the formation of a red or blue or purple color is an excellent presumptive test for an opium-related drug. This is positive also for most morphi-

nans, and some antihistamines can give a positive reaction. The formation of an orange or brown color is rather characteristic of phenethylamines and various amphetamine derivatives and compounds related to demerol.

Van Urk test. A mixture of a 2% ethanolic solution of para- dimethylaminobenzaldehyde (PDAB) and concentrated HCl. This is an excellent color test for the indolic 2-position hydrogen, and will give a distinct blue-purple color with very small quantities of indolic drugs (sub-microgram quantities). It is positive for other pyrroles and also tryptophane, but is usually employed for the DMT-psilocybin-LSD group, all indoles. A variant called the Gordon's test (with PDAB and 65% sulfuric acid can distinguish between LSD (blue) and the substituted tryptamines (yellow- green). In this, tryptophane gives no reaction.

Mecke test. In this, as with the following test reagents that contain sulfuric acid, it must be assumed (or determined) that the acid alone gives no response. The Mecke reagent is a solution of half a gram of selenous acid in 100 ml conc. sulfuric acid, and it is valuable both in its own right as well as in teasing apart the opiates that are positive in the Marquis test. Most of the group morphine-codeine-heroin give green or blue- green colors, but dilaudid is a yellow-green and PCP produces a yellow-orange color.

Froehde test. This reagent is a freshly prepared solution of 100 mg of sodium molybdate in 100 ml conc. sulfuric acid. It is a broadly applicable test giving positive tests with many drugs, often with a dramatic sequence of color changes seen. Morphine, for example, starts as violet then becomes, in sequence, green, brown-green, yellow, and finally ends up as a violet-blue. Codeine, on the other hand, starts a dirty green then goes through blue and ends up a light yellow. Very few colors are stable with Froehde's reagent, and careful attention must be paid to the test.

Mandelin test. This reagent is a solution of 1 g ammonium vanadate in 100 ml conc. sulfuric acid with or without the addition of a little water. The major opiates (morphine, heroin) give an immediate blue or brown color, but the relatives of methadone start with yellow or orange, and only slowly become deeply colored. Cocaine gives an orange color, and amphetamine and methamphetamine produce a green color.

These are the major tests, but there are literally hundreds, many for specific compounds, some for classes of drugs, and others for functional groups. An excellent reference is the Fifth Edition of the Merck Index which has some 4500 listed.

ILLEGAL MANUFACTURE OF DRUGS

In the area of criminalistics and crime lab work, the chemist is frequently called upon to assist law enforcement agents in the approach to and the securing of evidence from what may be an area of manufacturing of illegal drugs. First, a few minutes in the area of vocabulary.

Illegal. This term is explicit – it is an adjective that states that there is a law that proscribes something, and the thing referred to is that very something. In its more exact definition, it is something that is not allowed by law and so is often used to indicate that the something has not been specifically authorized. If there is an explicit pattern required by law (such as may be found in the rules of bridge or poker) then deviation from this pattern is illegal. If there is a pattern denied by law (the selling of heroin), then deviation is not illegal (which is not quite the same thing as legal). Legal, and not illegal, do not carry the same exact message to the court.

Illicit. This is a looser term for illegal, carrying the message that whatever might be referred to is not permitted. This denial may be that of law, but perhaps that of custom, of society, of church.

Clandestine. This term denotes secret or private. This is commonly applied to an action, a place, about which there is no public information. It may, of course, embrace illegal behavior, but it need not.

Private. Something intended for, or restricted to the use of a person or a group. There need be neither secretness nor illegality in it.

So, let's look at all this in light of the application of the adjective to a laboratory. First, there are no requirements of registration or of licensing of laboratories. And with no laws that dictate what a laboratory may or may not be, there can be no laboratory that is intrinsically illegal. There are many laws that dictate what may not be done in a laboratory, from the industrial safety laws for solvent storage to the drug laws for the manufacture of drugs, but the lab as such cannot be illegal. The similar argument applies exactly to the term "illicit laboratory." On the other hand, there is no question of the possible secretness of a laboratory or of the privateness of a laboratory as long as these words are seen in terms of a person's relationship to the lab. I alone may use it; it is private. I alone know of it; it is secret.

Another tricky bit of terminology is the use of strange adjectives in the description of drugs. Consider the term "illegal drug." If you investigate and try to describe a white powder which you know to be $C_{11}H_{21}NO_2HCl$, you may appreciate its infra-red spectrum, its melting point, its crystalline form or density, but there is no sense to a search into its being evil, or dangerous, or illegal, or even being a drug. All of these properties come from a person's interactions with the chemical, and are not

intrinsic in the chemical. There is, to my knowledge, no drug that is illegal any more than there is a drug that is legal. The laws can't apply. It is unlicensed or unauthorized possession of, or the making of, or the use of, or the selling of, or the buying of a chemical that is the illegal act. Or the illicit act. The next time you see headlines that say:

POLICE RAID ILLEGAL LAB
ARREST FOR ILLEGAL DRUG SALES
ACCUSATION OF DANGEROUS DRUG MANUFACTURING

be aware of the faulted thought processes at work.

At a less philosophical but more practical level, there are some generalities that apply to the investigation of laboratories where there may be illegal activity underway. For one thing one almost never finds a final product in any quantity. If there is the manufacturing of drugs for sale, then the money is the name of the game, and any salable product will be out and into profitable distribution as soon as it is made. True, there may be incriminating products as residues on filters, or on the mop in the corner, or in the garbage can, but the large quantities that make headlines are rarely there. What is more commonly found are the intermediates for manufacture, or the results of production that have gone wrong. Thus one can argue that there was the capacity for the production of $100,000 worth of methamphetamine a day, and the actual charges will finally be something like intent to manufacture, or conspiracy to manufacture. These are more difficult tasks for prosecution, but that is the DA's problem.

Your main concern is firstly the safety of you and your allies, and secondly the fair and appropriate collection of evidence.

Safety is an increasingly serious problem. PCP, for example, is highly volatile, and if one were to enter a closed room in which a quantity was lying about, or where a quantity had recently been stored, it is very possible to become seriously poisoned by it. A similar situation obtains with a laboratory where fentanyl or its analogs may be present. In this latter case this is due to its extreme potency. The inhalation of a milligram of dust may well be fatal in the absence of immediate medical action. An increasing number of law enforcement groups are now requiring respirators for entry.

Selection of evidence for eventual determination of the activity that had been underway is equally difficult. There is a bias easily (and even innocently) injected into the determination of the evidence chosen. If there are five reagent bottles present, which are labeled with contents that when combined could result in methamphetamine, the conclusions are pretty damning. If a thousand bottles are present, and 995 of them are unrelated to methamphetamine manufacture, the conclusions are proportionately more exculpatory. But if the chemist sees the 1000 bottles, and selects just the damning five, then there may be subsequent misinterpretation. This example is extreme, but it illustrates the way that bias can sneak in.

How do the police find laboratories where illegal activity may be underway? Almost always through informants. The will-call secretary at VWR Supply House has a phoned-in purchase order (for a cash sale) for a pound of mercuric chloride and 10 kilograms of phenyl-2-propanone. Thank you, sir, and it will be on the will-call dock tomorrow at 10:00 AM. And the person in the dirty white Camero on the other side of the parking lot the next morning is part of a team which will track the purchaser to his lair. Radio beepers are put in false bottoms of drums of anthranilic acid, if the purchase request is from an unknown customer. The person who sold you the needed starting chemicals and who offered to help you with the wet chemistry, was actually an undercover narcotics agent. The "little old lady" who lives upstairs and had rented the garage to those nice students, saw them running out of the garage coughing and choking at 3 AM followed by clouds of acrid smoke, and reported it to someone.

But knowing where a laboratory may be, and getting permission to enter and investigate are two very separate matters. A warrant for entry must be issued by a magistrate, and that can only be done following the presentation of sufficient evidence to support the argument that either a crime is being (or has been) committed, or that there is a threat to public safety. This often takes time and surveillance. Hence the documentation that 10 5-gallon carboys of ethyl ether are being stored in someone's kitchen may not be a sure clue that illegal activity is under way, but it is a clear threat to the neighbors. Or, by personal inspection (at a legal distance but in close proximity) there may the the smell of ether. And in many cases, the combination of "I detected the unmistakable smell of ether" and "In my experience, the smell of ether has always been associated with a meth lab" is a strong argument for getting a warrant signed. As a rule, one way or another, there is pretty good confidence of the nature of the activity under way well before the warrant is actually requested.

There is a close deductive relationship between the chemicals found in a laboratory or being sought, and the drug being manufactured. These are listed below with the major components grouped under each drug heading.

METHAMPHETAMINE (and amphetamine):

(1) Phenylacetone (phenyl-2-propanone, P-2-P) has classically been the major precursor of both amphetamine and methamphetamine. In fact the major commercial (and legal) use of this chemical is the manufacture of amphetamine. It has become very hard to find, however, especially since its listing as a Schedule II controlled substance in 1980. The preparation of phenylacetone itself has frequently been done. This starts from either phenylacetic acid or phenylacetonitrile (benzyl cyanide), neither of which are restricted by federal law. The weak but pervasive and distinct smell of phenylacetic acid is a frequently used factor in the request for the issuing of warrants. Once learned, it is unmistakable. The preparation of P-2-P involves additional reagents such as acetic anhydride and/or sodium acetate. The modification of phenylacetone can be exploited to synthesize a modification of amphetamine or methamphetamine. Thus the use of commercially available (and unrestricted) piperonylacetone (3,4-methylenedioxyphenylacetone) can lead to the manufacture of MDA or MDMA, both scheduled drugs.

(2) The amine component. For amphetamine, ammonia is needed, and for methamphetamine, methyl amine is needed. This latter chemical is rather closely watched, for although it has many commercial uses and is sold in volume to industry, it is nonetheless a necessary component for methamphetamine. It is sufficiently condemnatory when bought by an individual, that some efforts have been made to make it from yet other, more easily obtained, starting materials such as acetamide and bromine.

(3) The reducing agent. The reductive amination of P-2-P to the amine requires some form of hydrogenation. This may be catalytic (thus catalysts, and some form of hydrogen will be present), or involve borohydrides (currently $NaBH_3CN$ is most popular), or aluminum amalgam (the cheapest and the best). The latter requires aluminum foil (easy, the local supermarket and Reynolds Wrap) and mercuric chloride (difficult, a point of attention getting). All of these steps call for a common array of solvents and reagents (such as isopropyl alcohol and hydrochloric acid).

A somewhat related direction that has been encountered in the illegal preparation of methamphetamine has involved the reduction of the hydroxyl group of ephedrine (or of norephedrine to yield amphetamine). Here the usual process is to replace the hydroxyl group with a halide (perhaps with thionyl chloride) followed by reduction. A most current process avoids the use of any catalytic intermediary. The dissolving of ephedrine in concentrated hydriodide, preferably with the presence of red phosphorus, and a few days refluxing, very effectively reduces the ephedrine to methamphetamine. The phosphorus is optional, merely increasing the efficiency of the reduction. Extraction with Freon 11 eliminates the need of ether as a solvent. Dry hydrogen chloride gives the salt.

And, most recently there has been the appearance of N-methyl ephedrine in the illegal drug synthesis world. It's reduction produces N,N-dimethylamphetamine which has been found in the illicit drug community. Moves are afoot to make it illegal, although there is little data to define its pharmacological activity.

PHENCYCLIDINE (PCP) and close relatives:

(1) The amine component. Piperidine is the mandatory amine for phencyclidine, and it is a very closely watched chemical at both the state and the federal level. Although not a controlled chemical itself, it is explicitly illegal to possess it in a situation that can be construed to suggest the intention of manufacturing PCP. Substituted piperidines, pyrollidine, ethyl amine, methyl amine, and even ammonia, have been encountered in the illicit synthesis of PCP analogs.

(2) The aliphatic ring component. This is inevitably cyclohexanone. The commercial demand for cyclohexanone is so large, that there has been no effective effort to regulate this solvent. Cyclopentanone cannot be substituted in the chemical recipes for PCP in an effort to make an analog. The chemistry simply doesn't work.

(3) The aromatic ring component. This is usually bromobenzene, but

substituted bromobenzene derivatives, and other aromatic analogs (such as bromothiophene) have been seen.

(4) The displacable factor from the cyclohexanone-piperidine adduct intermediate. This is almost always an inorganic cyanide.

(5) The component needed to activate the aromatic ring. This is usually through a Grignard reaction (via the bromobenzene), and thus such things as magnesium and anhydrous ether are seen.

DIMETHYLTRYPTAMINE (DMT):

There are so many different procedures that might lead to DMT or analogs of DMT, that no one organization is useful. There must be a source of an indole ring (perhaps indole itself, or 4- benzyloxyindole, or indole-3-carboxaldehyde) but in some laboratories, even the indole ring itself is synthesized. The amines required are dimethylamine (for DMT or psilocin) or diethylamine (for DET). Other commonly encountered chemicals are:

Oxalyl chloride, for the attachment of the two carbon chain to the indole ring, and eventual amide formation with the above amines.

$LiAlH_4$ (for the reduction of these amides.

$POCl_3$ and DMF or N-methylformanilide, for the synthesis of aldehydes from indoles.

LYSERGIC ACID DIETHYLAMIDE (LSD):

The absolutely imperative and unavoidable starting material required for LSD is the lysergic acid ring system. This cannot be synthesized in any practical way, and so must come from natural sources. These are, ultimately, either the ergots from rye (and may be in the form of any of the many alkaloids present there, such as ergotamine or ergonovine) or from the Convolvulacaea seeds including the morning glories or baby Hawaiian wood rose (in the form of the simple amides of lysergic and isolysergic acid). The hydrolysis of these source materials yields lysergic (and isolysergic) acid, and this is the indispensable intermediate. LiOH is often used in this saponification step.

Diethylamine is a second component for which there is no substitution.

Finally there must be some way for the conversion of the acid to the diethyl amide, and here there are many options available. The activated intermediate can be made with sulfur trioxide, with trifluoracetic anhydride, $POCl_3$, hydrazine, and any of the many peptide synthetic reagents such as imidazole carbodiimide.

In most LSD laboratories, some form of chromatographic equipment is found, such as alumina or silica gel and some column for liquid chromatography. This is

needed to separate the synthesized LSD from the inevitable iso-LSD that is an inactive contaminant.

PHENETHYLAMINES

Again, the diversity of these compounds, and their related substituted amphetamine derivatives, is so great that no one listing can be adequate.

Most of these materials involve the conversion of an aldehyde to a nitrostyrene and its subsequent reduction. The aldehyde can be any one of dozens, involving methoxyl groups or alkyl groups, or even halides. The nitrostyrene is generated with either nitromethane or nitroethane, and frequently calls for the use of a catalyst such as ammonium acetate. The reduction step employs $LiAlH_4$ and this is probably the most consistent reagent found in such laboratories (and the most frequent source of fires and explosions).

Alternately, the amphetamine derivatives can be made from easily obtained essential oils such as safrole, employing an oxidant such as hydrogen peroxide or tetranitromethane, and finally ammonia.

The usual solvents (ether, THF, hexane, alcohol) and reagents (hydrochloric acid, sodium hydroxide, acetic acid) are encountered her, as in most laboratories.

HEROIN SUBSTITUTES

One of the most active areas of illegal syntheses at the present is in the area of MPPP, and meperidine analogs, and fentanyl and its analogs. Again, too diverse a group to easily organize. The usual amines encountered are methylamine (or N-methyl piperidinone) or in the case of fentanyl, aniline. Propionic anhydride is very common, but sometimes the more easily obtainable acetic anhydride takes its place. Ways of preparing the piperidine ring are seen, such as methyl acrylate and methyl methacrylate.

There is a large "etcetera" to be added here at the end. There are occasionally encountered special efforts to make specific drugs that are outside the main synthetic areas. Cocaine is often tried, but has probably never been successful. It is much more easily obtained from plant sources. Also the barbiturates are more easily stolen than made. Methaqualone (Ludes) are out of popularity at the moment, but here one can expect anthranilic acid and ortho-toluidine as the most frequent precursors. THC and the JB compounds are talked about, but rarely are there any serious efforts made to synthesize them.

A final reminder: Most labs are innocent. All are presumed to be, until found otherwise.

And in entering a strange laboratory, be safe, be observant, and be thorough.

PILLS, POWDERS AND EXCIPIENTS:

There is a logical and necessary process that is usually followed in the course of searching out the identity of evidence in a legal case. Each criminalistics lab will have its own variation, but one way or another, the following steps will be encountered.

(1) Receiving the alleged drug to be evaluated:

A very necessary consideration that must be thought about before any time or effort is invested, is the chain of possession that brought the sample to you.

At the far end of the chain, the origin of the seizure, there must be a person who gathered this evidence, and who will be able to sit on the stand under oath and tell the court, yes, this is the sample I took into my possession, and then I gave it to Jones. He knows because his initials are on it and he has a receipt from Jones. And you may be that original person, and might entrust the evidence to others until it comes back to you in the lab. The sample coming to you within the lab must have an adequate seal, be adequately identified, and be delivered to you by an identified person. I have seen evidence envelops with a dozen cuts, tapes, reseals – all of which are initialed and dated.

Two times I have received samples that were improper – one, a blood sample for a verification of a blood alcohol level at the request of the defendant's lawyer, the other a sample of alleged marijuana leaves for THC validation. The first had only the name of the arresting officer on the vial (i.e., it was anonymous) and the second was in an unsealed paper bag. I refused to analyze either one, and both refusals stood up in court.

(2) Fairness of sampling:

This is a perpetual problem in any analytic work: How can one take a representative sample for analysis? With gas samples there is no problem, with liquid samples there rarely is a problem, but with solid samples there is no completely satisfactory solution. A fundamental axiom – the coarser and more heterogeneous the sample, the less reliable the result.

Ideally, one should consume the entire sample, which of course resolves the conflict introduced by the heterogeneousness. But two problems arise: (1) no sample is left over further analysis, and some states (such as California) have judgments that have gone against the prosecution (I believe the original ruling was called the Hitch decision) when there has been no sample available to the defendant for an independent analysis by a second lab, and (2) with a large sample the handling problem can become ludicrous. Consider the seizure of a 16 foot high marijuana plant, roots, mud ball, stems, leaves, and a small osprey nest at the top, all weighing some 250 lbs, and

largely hanging off the back of the sheriff's pickup truck in the parking lot in front of the lab. There is no possible way to take a "representative" sample because only part of it could ever be retained, and any move you make will reflect some bias you hold, at some level. Even if you cut the monster down the middle, the two haves would certainly not be identical, and the basic questions is still unanswered. And unanswerable.

I was involved in a seizure of something over a hundred marijuana plants in a Humboldt County case about three years ago. The sheriff's deputy logged the samples seized, and took an occasional "representative" leaf from the plants as they passed *him* on the way to the flat-bed truck which, in turn, took them on the the local incinerator. By the time the defense attorney was finished with cross examination (how many plants – about 120 or so; did you tally them – no; what was the weight – maybe 3 thousand pounds or so; did you weigh them – no; how many leaves did you collect – a bag full; how did you choose the leaves – one from here and one from there; did you collect stem, seed, mud, etc. – no; how did you know they were marijuana plants at the time – I recognized them as such; how did you learn to identify marijuana plants by sight – I took an all day course in drug identification when I joined the force; could there have been a tomato plant amongst them – I'm not sure).

The judge threw out this entire portion of the testimony.

(3) Documentation of evidence:

When a sample is in hand at the laboratory, do all potentially needed documentation before it is damaged or modified in any way. Weigh it, describe it, photograph it if needed, write down a description of your impressions – any of this that might be useful at some later date. If there is any indication that it was found in association with something else (in, around, under, part of) get this recorded while it is still fresh in mind. Frequently the incriminating evidence can be the white smudge on the inside of the Buchner funnel, or the dust on the blade of the kitchen fan.

(4) What is the question:

Then, before any work is done, be sure of just what question has been asked of you. The most straightforward one is – Is there such-and-such present? And if so, how much or how pure? But as discussed in the earliest lectures, beware the traps that are associated with a fishing question – What is present?

The question of purity is often brought up. Say a sample is reasonably pure cocaine. You may be asked just how pure is it? This is usually a ploy so that the quality and thus the believability of your work will be more impressive to the jury. In the narcotics officer's philosophy, it is sometimes suggested that the effectiveness of enforcement work is demonstrated by the scarcity of a drug (the better the police action, the harder the drug is to find) and so if there is a decrease in purity it can be used as demonstration that the "war against heroin" (so to speak) is being won. But in the criminalist's lab, the only merit is to tell a strong form from a trace component.

But, the purity report is tricky. Take the example of cocaine. Cocaine as the free base has a molecular weight of about 303, and as the hydrochloride, a molecular weight of about 340. The Federal drug schedules identifies it as a component of the Coca plant. If you have a sample of pretty pure cocaine hydrochloride, then using cocaine as a reference standard, it is less than 90% pure. It obviously contains some 10% (by weight) of hydrochloric acid, which is not a scheduled chemical. And the reverse becomes even stranger, in that if cocaine hydrochloride is your standard, then cocaine base can be over 110% in purity!

A more general problem associated with purity must also be considered. The statement of the exact purity of a substance cannot be made without identifying either the method of measurement or the nature of the impurity. If you put a sample in the GC and integrate all peaks coming out, then the proportion of the area of the peak of the desired drug to the summed area of all peaks will be the percentage purity. The method has been identified, and one can report that the submitted sample was cocaine base with a purity of 97.37%, as measured by gas chromatography. If, on the other hand, one assumes that the impurity is the opposite isomer (i.e., the sample is racemic cocaine without optical rotation) then one can state that the submitted sample was cocaine base with a purity of about 50%, as measured by polarimetry (the impurity is specified). 100% purity does not exist.

(5) If your sample is a nice solid, then – – :

Such a sample has usually come from some territory associated with people at or about the retail level. There might be a capsule, a powder in a folded paper, or a tablet. If one is dealing with a single dosage form, it is worth the trouble to try to determine if the sample is largely pure drug or largely excipient. The term excipient refers to a (presumed) inactive diluter, used to add bulk to the drug to either increase the ease of subdividing or to dilute the sample to extend its apparent quantity.

A most direct manner to make this distinction is the infra-red spectrometer. Most laboratories will have acquired an atlas of infra-red spectra of common excipients, and often this can serve as a quick establishment of the nature of the diluting but can also serve as a fingerprint of its origin. If the sample is in a capsule, after having weighed, measured, described, etc., empty it and record the net weight. Do not discard anything. One recent problem came up in an analysis where the criminalist carefully emptied the capsule, saved the solids and discarded the capsule body. The solids proved to be a mixture of sugars, but there was no drug apparent. Subsequently, it was shown that the drug had been LSD and had been applied, apparently in a droplet of concentrated volatile organic solution, to the inside of the capsule which had only subsequently been filled with inactive sugars. The actual drug had been been discarded.

Powders in paper are handled the same way. Tablets are treated somewhat differently. Here it is most desirable to measure all aspects of the tablet, in that there is a collection of reference dimensions that can make a connection, using small marks

and fine dimensional difference, between a tablet and the press that made it. This art is called "ballistics" because of its obvious parallel to rifling markings on bullets. Also, a tablet is usually much more heterogeneous than a powder. It may be layered, there may have been surface oxidation or moisture exposure, and there is almost certainly one or more excipients present. These I usually divide in half (saving one half) and then subdivide the remainder, eventually pulverizing it.

The usual excipients encountered are things that can be purchased easily and without gaining attention, and which will not interfere with the action of the drug. Sugars are common, and one sees sucrose, lactose, and mannitol most commonly. Inorganic salts that are not basic are often seen, such as the phosphates, sulfates, or silicates. Watch for things like commercial fertilizers (calcium phosphate) which can be bought in bulk easily. I have encountered such things as boraxo, milk of magnesia, and talc. When a powder might be readied for injection (as with heroin) a thoughtful dealer will keep the excipient water soluble (as with a sugar) but sadly many dealers are not thoughtful and the damaging injection of insoluble particulate matter is one of the risks of using street heroin.

If the sample is largely some organic salt, or in other words, largely drug, often a referral to the reference library can give a good clue to its identity. Only a small portion of it is needed for further analysis (through GC, GCMS, UV, whatever is appropriate). But if the sample is largely excipient, then much of it may be needed to do the analysis, as some extraction step is usually needed next. The procedure tree to be followed is usually established in each laboratory, and after even a few cases, you will begin to develop your own patterns.

(6) But if the sample is a goop, then -- :

Here one finds plant material, solutions of stuff in dirty bottles, smudges and stains from the lab evidence, or almost empty containers. There is no "best" way to proceed. Some will try for an early extraction to get a base fraction for GC or color tests. Some take advantage of the fact that methanol will almost always extract some drug from a dried sample, and this methanol extract can be surveyed by TLC. If the original dried sample is mixed with dry base (such as sodium carbonate) then this methanol extract can be surveyed by GC as well. Many laboratories have a broad survey screening chart, showing where some hundred or hundreds of drugs show up on a general TLC run or a general GC run.

It is so much nicer when the boss asks you if a given drug is present!

MARIJUANA, COCAINE AND HEROIN (the three big ones):

Although there are many score drugs entered into the schedules of the Controlled Substances Act, in practice the Federal authorities are most concerned with these three drugs only. This is probably due to the fact that all of these are involved in complex money transactions, and the transfer of money, the making of money, the power associated with money, is the commanding factor in the DEA's priorities. It is certainly true that there are cases that involve LSD, and peyote, and *Psilocybe* mushrooms, but they have neither the press appeal nor the public response satisfaction that can be found with one of the three big ones.

I would like to take each of these three in turn, giving some additional in-depth information that might be of value if you are ever on the stand as an expert, and get some odd-ball question thrown at you.

MARIJUANA:

This is the common name for the plant that is legally define by the binomial *Cannabis sativa.* It is believed by many to be monotypic.

Vocabulary. Anyone who has been in and about the area of biology has encountered the term "binomial" but this is the kind of question that you may have to answer under pressure. After centuries of efforts to classify plants and animals in some accurate way, it remained for Linnaeus, in the eighteenth century to establish a formula that has, with many modifications, endured. The current form of his scheme employs a two-word name to refer to a plant or animal that is an individual that can successfully breed with its like. The two words are the Genus (the grouping of animals or plants being considered) and the species (the interbreeding class). These two words together are used to name a animal or plant, not as an individual but as a member of a identified and identifiable group, and together are call the binomial name for this group.

The three-species defense:

Monotypic refers to a Genus which contains only one species. The laws that proscribe marijuana equate it to the binomial *Cannabis sativa* and it has been accepted that the Genus *Cannabis* has but one species.

The three-species defense has been raised in cases where this equation has been challenged. There are many reports in the botanical literature that claim other species, such as *C.indica* and *C. ruderalis*, and since the law had defined marijuana rigidly as *C. sativa* the defense strategies become obvious. A general rule of botany is that one must inspect the blossom of a plant to make an exacting identification. And since the blossom of marijuana is not the form usually presented into evidence, the

calling of an expert botanist as an expert witness can sow confusion into questions of plant identification. There are many variants of marijuana, and some of them may show leaves of most unusual character.

There have been many fascinating trials that have explored this point, but at the present time the judicial consensus is that the intent of the writers of the law was marijuana as being controlled, and the other names are simply inexact synonyms for it.

Identification of marijuana for legal purposes:

The identification of marijuana for legal purposes is needed only when the charge has been the possession of marijuana. Remember, this is the plant, and not the component chemical. There are three levels of drug, here, and only two levels of law. There is the plant, marijuana, and it is named explicitly in the law. There is THC (to be discussed below) and it is a major active component of the plant, but it is explicitly named by chemical structure. However, extracts of the plant (such as red oil), or physical processing fractions of the plant (such as the resin, or hashish) are neither plant nor chemical. This is touchy territory legally.

How is the plant identified as being marijuana? There are both morphological (morphology, the knowledge of form) and chemical procedures that are accepted.

The marijuana plant is dioecious (two houses, i.e., there are separate male and female plants) and is best superficially recognized by its fan-shaped leaf structure, with each fan containing an odd number of serrated leaves.

A microscopic examination of the under surface of the leaf will show small, tear-drop shaped structures known as cystolyths (cystolyth, a sac of stones). These are unusual in that they actually contain CaCO3 and will show efferverscence on treatment with dilute acid. A comparison with a documented sample of marijuana is needed for comparison, and some experience with other cystolith-carrying plants is very desirable.

Historically, there are three color tests for marijuana. All are run on hydrocarbon extracts of the suspected leaf material, either on the hydrocarbon itself or on the residues left after evaporation. The Beam test employs a 5% ethanolic KOH solution and produces a purple color. It's rather specific, and depends on the presence of hydroxyquinones generated from cannibidiol (a marijuana component) by air oxidation in the strong base. The Ghamravy test employs a heated mixture of extract of alleged marijuana leaf, and a reagent containing p-dimethylaminobenzaldehyde and slightly diluted sulfuric acid. A positive reaction is the development of a red-brown color which turns purple red on cooling and then blue or indigo on subsequent dilution with water. This test is very sensitive, but rather general (similar colors with any plant containing phenolics or certain terpene aldehydes. Neither test is much used today.

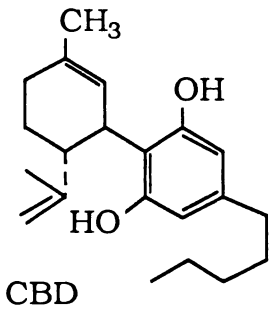
The third color test, the Duquenois, is broadly used currently and is generally accepted as an excellent presumptive test. A test solution containing vanillin and acetaldehyde in ethanol is added to the hexane extract, followed by concentrated HCl. The generation of a violet color is considered positive for marijuana. The Levine modification involves the extraction of the colored factor from an aqueous layer into chloroform, and is claimed to increase the specificity of the test. The test gives false positives with several resorcinol-like compounds, but the colors generated are usually different.

Chromatography of extracts from the alleged illegal leaf is required for the confirmation of any of the above presumptive tests. Thin-layer chromatography is frequently used, exploiting the phenolic nature of the components in the use of a spray reagent of high sensitivity. Fast blue salt B (0.1% in 1 N sodium hydroxide, freshly prepared) will give purple to red colors with the three major marijuana components, THC, CBD and CBN (see below). The sensitivity is in the sub-microgram area. The use of gas chromatography is now more common, and not only are the major components characterizable by retention times, but considerable success has been made in the assignment of geographic origins, by the overall chromatogram finger-print.

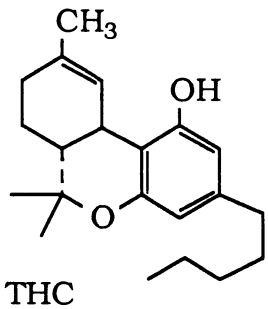
Remember that in any and all analyses, reference samples (called exemplars) must be at hand, against which comparisons are to be made.

Chemical components of marijuana:

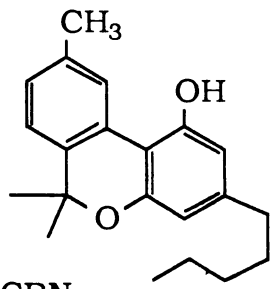
The three components of classical importance in marijuana are cannabidiol (CBD), tetrahydrocannibinol (THC) and cannibinol (CBN). There structures are given.



CBD



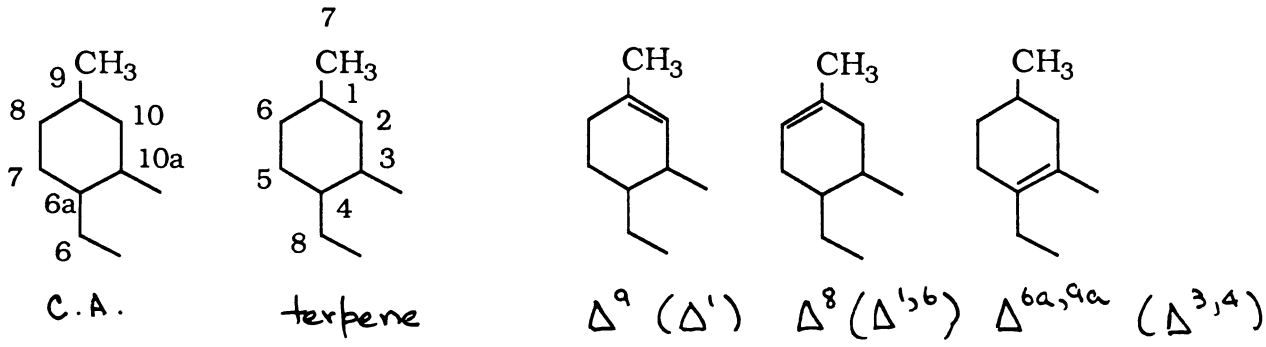
THC



CBN

The flow of biosynthetic chemistry in the plant is from left to right; the diol (CBD) is cyclized to the three-ring THC, which is eventually degraded (UV light, air-oxidation) to the aromatic CBN. The component generally held responsible for the CNS action of marijuana is THC. The major form in the plant is called Δ^9 -THC (or Δ^1 -THC, with a different numbering system) although the CNS-active isomer Δ^8 (or $\Delta^{1(6)}$) is also

present. Both of these isomers (as well as the unnatural $\Delta^{6a,10a}$, or $\Delta^{3,4}$ isomers) are explicitly named as Schedule 1 drugs in the CSA. The numbering systems of the THC molecule are weird. There is a Chemical Abstracts system, that depends on the entire three-ring system of the THC molecule. With it, the inner carbon atoms must be named 6a and 10a for the location of a double bond. The terpene system is straightforward, but it does not acknowledge an aromatic ring. These, and the double bond names, are shown below.



The diol (CBD) is of low pharmacological activity, and is the major component of marijuana raised primarily for fiber. It can be chemically cyclized to THC by acid catalysis, however, and this is the basis of the "Isomerizer" craze in the head shops of a few years ago.

A recent issue of the Federal Register (October 16, 1985) has announced the proposed rescheduling of Δ^9-THC from Schedule I to Schedule II so that it may be prescribed under the name of Dronabinol for the treatment of nausea and vomiting associated with cancer chemotherapy in certain patients. A specific form only (in sesame oil and in soft gelatin capsules) is to be reassigned to Schedule II; the uncompounded drug remains a Schedule I substance.

COCAINE

Cocaine, its importation, sale, use, and abuse, represent one of the major drug problems in the country today. And it represents a major target for the efforts of the law enforcement groups. For practical purposes, all of the illegally used cocaine comes in from overseas. There have been many attempts to achieve total synthesis for commercial purposes (leading to a racemate) and studies have reported possible impurities that would serve as markers for such actions. However, by far, most commercial cocaine comes from plant sources, although there is often a partial resynthesis of the alkaloid from the ecgonine obtained from the hydrolysis of the alkaloidal by-products.

Plant origins:

All commercial cocaine comes from one of the other of the coca plants *Erythroxylum coca* or *E. novogranatense*. The leaves of these plants have been chewed

by South American Indians for centuries to alleviate hunger and increase endurance. The plant contains some twenty additional alkaloids, but cocaine is the principal one, being present to about 1.5% of the weight. Although in the wild, the plants may grow to 12 to 18 feet in height, under cultivation it is held at about six feet, and will live perhaps 50 years.

Preparation and usage:

A mixture of coca leaves and sodium carbonate is ground under some hydrocarbon (such as gasoline) and stirred for two or three days. The liquid is poured off and treated with sulfuric acid, which precipitates all alkaloids as the sulfate. The overhead fluid is decanted, these salts dried and compressed into 1 Kg. bricks. This is called pasta, and has the appearance and texture of portland cement. In this intermediate form it is smoked (admittedly with much inefficiency, being largely salt and thus much pyrolysed), sometimes mixed with tobacco or marijuana (called basucos and pitillos), and sometimes treated with a little ammonia water to give a sort of "free base." In this form, the user is exposed to the entire spectrum of alkaloids originally present in the plant.

The pasta is redissolved in acetone, filtered, and treated with hydrochloric acid in ethanol, to yield "clorhidrato" which is dried by heat. There are many variations on this process but the flow scheme, isolate as sulfate, purify as hydrochloride, is maintained. With the current efforts to restrict the flow of processing chemicals into certain countries in South America, there is an increasing quantity of pasta appearing in the United States in its raw and smokable form.

d-Cocaine loophole:

The federal law regulating cocaine is worded in a way that implies that only the l-isomer of cocaine is scheduled. It states that the material from the coca plant is scheduled, and the coca plant produces only the levo-isomer. Thus it is necessary to obtain laboratory evidence that a seized sample contains the l-isomer. This is a procedure that requires extra steps, and is being sidestepped by the passage of laws (as in California April 2, 1985) which makes synthetic cocaine illegal as well as natural cocaine. This effectively puts d-cocaine onto the legal books and circumvents the need of determining optical properties. A recent change in Federal Law now specifies both the optical and the geometric isomers of cocaine as being scheduled. Thus the unnatural form, as well as the allo- and the pseudo- forms, are covered by law.

Color tests:

There are a number of presumptive color tests advertised for the detection of cocaine, but only two appear to have merit. One of these is used largely in the home market; is the is clorox test. A light sprinkling of alleged cocaine is added to a glass of clear clorox. It will drop quickly in streamers leaving a whitish tinge behind. Both Lidocaine and procaine stay on the surface with the formation of yellow to brown to red colors. Amphetamine also stays on the surface and does not discolor.

The cobalt thiocyanate test for cocaine (or the Ruybal, or the Scott, test) is the most reliable and is used in many field analyses as a presumptive test. A solution of 2% cobaltous thiocyanate is dissolved in water, and then diluted 1:1 with 96% glycerin. To this a small amount of alleged cocaine is added, and if the solution turns blue, there may be cocaine present. The addition of a drop of conc. HCl should convert the blue to a light pink color which, upon shaking with chloroform, should extract the original blue into the organic layer. If the test fails, there is probably no cocaine present, but if it succeeds, there may be false positive drugs present such as lidocaine, procaine and benzocaine. Most other synthetic local anesthetics give no response.

There has been quite a bit of publicity given recently to crack, as if it were different from cocaine. The penalties may be more severe, and the social hazard might possibly be so, too. But chemically, the two are identical. From the strictly forensic point of view, how could one determine if a smudge is cocaine salt or cocaine free base (which is identical to crack)? The best single tool would be the pH meter. If it is up in the pH 9 to pH 10 area (or higher) it is crack (free base). If is is around pH 6 or below, it might well be cocaine hydrochloride. Remember, human sweat can be quite basic.

HEROIN

Heroin is one of the earliest and the most socially disruptive of the drugs of abuse. In the early decades of the century, there was little distortion that could enter the heroin picture — a white crystalline solid that gave a positive Marquis test was certainly heroin.

Initially heroin, the diacetyl ester of morphine, was introduced into medicine as a safe substitute for patients who were dependent on morphine. The really strong start that morphine had in the drug scene in this country was a consequence of the introduction of the hypodermic needle and syringe during the civil war. Morphine was heavily used there on both sides, and many of the veterans of this conflict maintained a strong dependence on it. After that war it slipped more and more into being a "woman's problem" with the broad distribution of patent medicine, and it became a western concern with the importation of many Chinese laborers destined for the transcontinental railway.

The problem today is ever so much more complex. Heroin is sold, not as the chemical with proper structure, but as the chemical with an acceptable pharmacological effect. If a particularly popular brand or strain of heroin becomes popular, then that name applies to anything that carries the heroin response. This may be China White or Mexican Brown. There are two very popular and complex areas of misrepresentation currently seen in the drug traffic. They are considered separately.

Morphine substitutes:

Many man years have been invested in the methodical changing of the morphine structure, in the hope that the analgesic action could be separated from the "euphoric" action. It was found that the complex ring system that characterized the

opiates could be dropped, and the simpler compounds that remained might well maintain the analgesic virtue of morphine. But the abuse aspect of the compounds remained as well.

A moment is needed to give what is probably the legal distinction between two terms which are never explictly defined in the written law. Opium derivatives and opiates. Opium derivatives collectively represent those drugs that depend to some explicit measure on opium, or some compound from opium, for their manufacture. The usual chemical intermediate here is thebaine, although morphine itself can serve for several derivatives.

Opiates, on the other hand, generally represent drugs that have the pharmacological properties of some of the opium alkaloids, but which are synthesized in the laboratory totally independent from any plant origin.

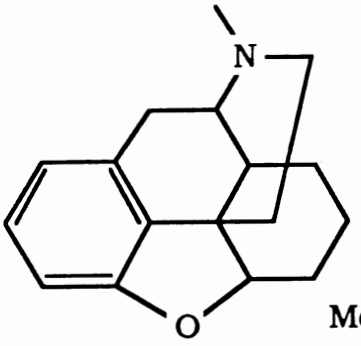
The flow diagram on the next page gives this evolution from the five-ringed structure of morphine down to the simple piperidine nucleus, and even open-chain analogs that are quite effective as analgesics. On the first line, the elaboration of morphine into a six-ring nucleus via a Diels Alder condensation, gives rise to a class of compounds known as the Oripavines, or the Bentley compounds. These are extraordinarily potent narcotic-analgesics, and provide the "tranquilizers" that are used to stun wild animals at a distance with darts. The potency in man, as narcotics, is in the small microgram level.

Much publicity has been given recently to two classes of heroin substitutes, the MPPP family and the fentanyl analogs.

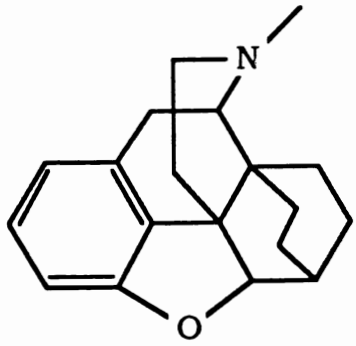
MPPP

The very simple structure of Meperidine (see next page) has prompted much research into variants and modifications that might retain the biological potency but decrease abuse potential. The oxygen function at the piperidine 4-position must be maintained, but there is little difference between an ester (COO as shown), a reversed ester (OCO) or even a ketonic carbonyl. A large number of compounds within each of these families have been explored pharmacologically, and have been in the patent and scientific literature for perhaps 20 years.

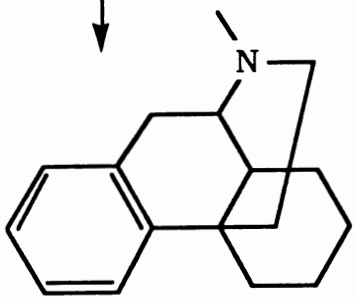
Periodically, the reversed ester structure (MPPP) has appeared on the street, sold as heroin. Recently, a batch was prepared in which the intermediate alcohol was overheated in the attempt to prepare the propionate ester, and dehydration occurred to form the unsaturated analog, MPTP. This material had no narcotic properties but, rather, had a burning sensation when injected and produced a light-headedness. But the tragic part of the story is that the users came down with irreversible Parkinson's disease. Here is a handful of known victims, and perhaps hundreds who have been exposed, and are potentially subject to early onset of the disease. The post-mortem of one victim showed morphological changes in the brain (in the substantia nigra) that were identical to those who suffered from the disease spontaneously. The experimen



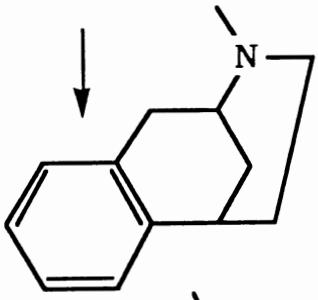
Morphine



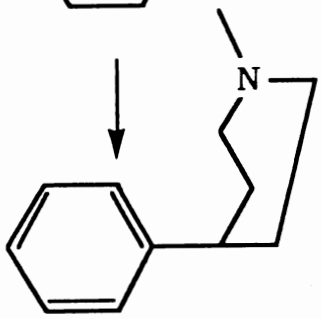
Bentley Compounds



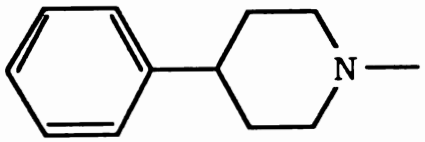
Morphinan



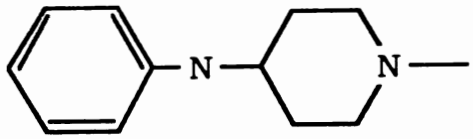
Benzomorphan



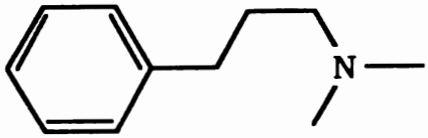
or



Meperidine



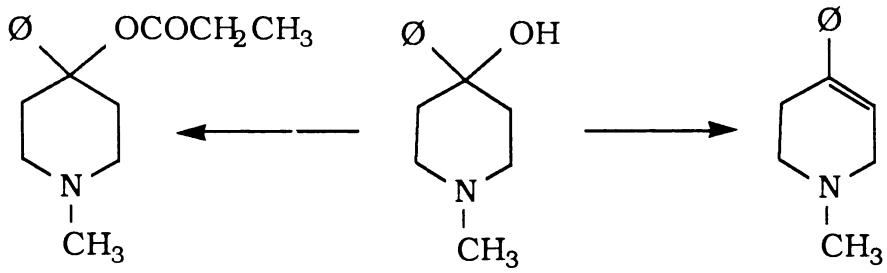
Fentanyl



Methadone

The simplification progression from morphine, bond by bond, to the open-chain methadone systems. These are skeletal structures, not complete structure.

tal branch of science has now available an excellent model for Parkinson's disease (with the use of MPTP in primates, it is not neurotoxic in any lower animal) but at a tragic cost.



MPPP MPTP

Fentanyl

A second compound of current notoriety is Fentanyl. Actually, the narcotic Fentanyl has been used in surgery as an analgesic for many years, but its high potency and relative ease of synthesis has provoked its appearance on the streets as a substitute for heroin. It is sold under names that imply it is heroin (China White, Persian White, etc.) and the user (and possibly the seller, too) is unaware of its true identity.

But the structure of Fentanyl allows many minor modifications, and many of these have been exploited in making variants, which maintain the potency and action (with qualitative changes) but which are not explicitly covered by the drug laws. There has been a cat-and-mouse game under way for several years. The enactment of the Analogue Drug Ac5 was an attempt to find some legal control.

PUBLIC LAW 91-513

THE COMPREHENSIVE DRUG ABUSE PREVENTION AND CONTROL ACT OF 1970

This is a transcription of the listings of scheduled drugs in Public Law 91-513. On the left is the exact text from the first printing, published October 27, 1970. On the right is the exact text taken from the April 1, 1990 edition of the Code of Federal Regulations, the most recent copy available at the time of this compilation. The drugs added to the law with PL 101-647 (1990) have also been entered, bringving this list current (April, 1991). The two listings have been juxtaposed, and the appropriate text spread out to correspond to the writing currently in effect. This allows an easy location of the additions, deletions and textual changes that have occured over the intervening two decades..

TEXT OF OCTOBER, 1970

Schedule I

(a) Unless specifically excepted or unless listed in another schedule, any of the following opiates, including their isomers, esters, ethers, salts, and salts of isomers, esters and ethers, whenever the existence of such isomers, esters, ethers, and salts is possible within the specific chemical designation:

(1) Acetylmethadol
(2) Allylprodine
(3) Alphaacetylmathadol (sic)
(4) Alphameprodine
(5) Alphamethadol

TEXT OF APRIL, 1991

§ 1308.11 Schedule I

(a) Schedule I shall consist of the drugs and other substances, by whatever official name, common or unusual name, chemical name, or brand name designated, listed in this section. Each drug or substance has been assigned the DEA Controlled Substance Code Number set forth opposite it.

(b) *Opiates*. Unless specifically excepted or unless listed in another schedule, any of the following opiates, including their isomers, esters, ethers, salts, and salts of isomers, esters and ethers, whenever the existence of such isomers, esters, ethers, and salts is possible within the specific chemical designation:

(1) Acetyl-alpha-methylfentanyl (N-[1-(1-methyl-2-phenethyl)-4-piperidinyl]-N-phenyl-acetamide9815
(2) Acetylmethadol 9601
(3) Allylprodine 9602
(4) Alphaacetylmethadol 9603
(5) Alphameprodine 9604
(6) Alphamethadol 9605
(7) Alpha-methylfentanyl (N-[1-(alpha-methyl-beta-phenyl)ethyl-4-piperidyl] propionanilide; 1-(1-methyl-2-phenylethyl-4-(N-propanilido)piperidine 9814
(8) Alpha-methylthiofentanyl N-[1-

	methyl-2-(2-thienyl)ethyl-4-piperidinyl]N-phenylpropanamide)	9832
(6) Benzethidine	(9) Benzethidine	9606
(7) Betacetylmethadol	(10) Betacetylmethadol	9607
	(11) Beta-hydroxyfentanyl (N-[1-(2-hydroxy-2-phenethyl-4-piperidinyl]-N-phenylpropanamide	9830
	(12) Beta-hydroxy-3-methylfentanyl (other name: N-[1-2-hydroxy-2-phenethyl)-3-methyl-4-piperidinyl]-N-phenylpropanamide	9831
(8) Betameprodine	(13) Betameprodine	9608
(9) Betamethadol	(14) Betamethadol	9609
(10) Betaprodine	(15) Betaprodine	9611
(11) Clonitazine	(16) Clonitazine	9612
(12) Dextromoramide	(17) Dextromoramide	9613
(13) Dextrorphan		
(14) Diampromide	(18) Diampromide	9615
(15) Diethylthiambutene	(19) Diethylthiambutene	9616
	(20) Difenoxin	9168
(16) Dimenoxadol	(21) Dimenoxadol	9617
(17) Dimepheptanol	(22) Dimepheptanol	9618
(18) Dimethylthiambutene	(23) Dimethylthiambutene	9619
(19) Dioxaphetyl butyrate	(24) Dioxaphetyl butyrate	9621
(20) Dipipanone	(25) Dipipanone	9622
(21) Ethylmethylthiambutene	(26) Ethylmethylthiambutene	9623
(22) Etonitazine	(27) Etonitazine	9624
(23) Etoxeridine	(28) Etoxeridine	9625
(24) Furethidine	(29) Furethidine	9626
(25) Hydroxypethidine	(30) Hydroxypethidine	9627
(26) Ketobemidone	(31) Ketobemidone	9628
(27) Levomoramide	(32) Levomoramide	9629
(28) Levophenacylmorphan	(33) Levophenacylmorphan	9631
	(34) 3-Methylfentanyl (N-[3-methyl-1-(2-phenethyl)-4-piperidyl]-N-phenylpropanamide	9813
	(35) 3-Methylthiofentanyl (N-[(3-methyl-1-(2-thienyl)ethyl-4-piperidinyl]-N-phenylpropanamide)	9833
	(36) Morpheridine	9632
	(37) MPPP (1-methyl-4-phenyl-4-propionoxypiperidine)	9661
(29) Morpheridine	(38) Noracymethadol	9633
(30) Noracymethadol	(39) Norlevorphanol	9634
(31) Norlevorphanol	(40) Normethadone	9635
(32) Normethadone	(41) Norpipanone	9636
(33) Norpipanone	(42) Para-fluorofentanyl (N-(4-fluorophenyl-N-[1-(2-phenethyl)-4-piperidinyl]-propanamide	9812
	(43) PEPAP (1-(2-phenethyl)-4-phenyl-4-propionoxypiperidine)	9663
(34) Phenadoxone	(44) Phenadoxone	9637
(35) Phenampromide	(45) Phenampromide	9638
(36) Phenomorphan	(46) Phenomorphan	9647

(37) Phenoperidine
(38) Piritramide
(39) Proheptazine
(40) Properidine

(41) Racemoramide

(42) Trimeperidine

(b) Unless specifically excepted or unless listed in another schedule, and of the following opium derivatives, their salts, isomers, and salts of isomers whenever the existence of such salts, isomers, and salts of isomers is possible within the specific chemical designation:

(1) Acetorphine
(2) Acetyldihydrocodeine
(3) Benzylmorphine
(4) Codeine methylbromide
(5) Codeine-N-Oxide
(6) Cyprenorphine
(7) Desomorphine
(8) Dihydromorphine

(9) Etorphine

(10) Heroin
(11) Hydromorphinol
(12) Methyldesorphine
(13) Methylhydromorphine
(14) Morphine methylbromide
(15) Morphine methylsulfonate
(16) Morphine-N-Oxide
(17) Myrophine
(18) Nicocodeine
(19) Nicomorphine
(20) Normorphine
(21) Pholcodine
(22) Thebacon

(c) Unless specifically excepted or unless listed in another schedule, any material, compound, mixture, or preparation which contains any quantity of the following hallucinogenic substances, or which contains any of their salts, isomers, and salts of isomers, whenever the existence of such salts, isomers, and salts of isomers is possible within the specific chemical designation:

(the order has been upset to allow exact correspondence betweenthe two texts)

(47) Phenoperidine 9641
(48) Piritramide 9642
(49) Proheptazine 9643
(50) Properidine 9644
(51) Propiram 9649
(52) Racemoramide 9645
(53) Thiofentanyl (N-Phenyl-N-[1-(2-thenyl)-ethyl-4-piperidinyl]propanamide 9835
(54) Tilidine 9750
(55) Trimeperidine 9646

(c) *Opium derivatives.* Unless specifically excepted or unless listed in another schedule, and of the following opium derivatives, its salts, isomers, and salts of isomers whenever the existence of such salts, isomers, and salts of isomers is possible within the specific chemical designation:

(1) Acetorphine 9319
(2) Acetyldihydrocodeine 9051
(3) Benzylmorphine 9052
(4) Codeine methylbromide 9070
(5) Codeine-N-Oxide 9053
(6) Cyprenorphine 9054
(7) Desomorphine 9055
(8) Dihydromorphine 9145
(9) Drotebanol 9335
(10) Etorphine (except hydrochloride salt) 9056
(11) Heroin 9200
(12) Hydromorphinol 9301
(13) Methyldesorphine 9302
(14) Methyldihydromorphine 9304
(15) Morphine methylbromide 9305
(16) Morphine methylsulfonate 9306
(17) Morphine-N-Oxide 9307
(18) Myrophine 9308
(19) Nicocodeine 9309
(20) Nicomorphine 9312
(21) Normorphine 9313
(22) Pholcodine 9314
(23) Thebacon 9315

(d) *Hallucinogenic substances.* Unless specifically excepted or unless listed in another schedule, any material, compound, mixture, or preparation which contains any quantity of the following hallucinogenic substances, or which contains any of its salts, isomers, and salts of isomers, whenever the existence of such salts, isomers, and salts of isomers is possible within the specific chemical designation (for the purposes of this paragraph only, the term "isomer" includes the optical, positional and geometric isomers):

	(1) 4-bromo-2,5-dimethoxy-amphetamine 7391 Some trade or other names: 4-bromo-2,5-dimethoxy-alpha-methylphenethylamine; 4-bromo-2,5-DMA
	(2) 2,5-dimethoxyamphetamine 7396 Some trade or other names: 2,5-dimethoxy-alpha-methylphenethylamine; 2,5-DMA
	(3) 4-methoxyamphetamine 7411 Some trade or other names: 4-methoxy-alpha-methylphenethylamine; paramethoxyamphetamine, PMA
(2) 5-methoxy-3,4-methylenedioxy amphetamine	(4) 5-methoxy-3,4-methylenedioxy-amphetamine 7401
(7) 4-methyl-2,5-dimethoxy-amphetamine	(5) 4-methyl-2,5-dimethoxy-amphetamine 7395 Some trade or other names: 4-methyl-2,5-dimethoxy-alpha-methylphenethylamine; "DOM"; and "STP"
(1) 3,4-methylenedioxy amphetamine	(6) 3,4-methylenedioxy amphetamine 7400
	(7) 3,4-methylenedioxymethamphetamine (MDMA) 7405
	(8) 3,4-methylenedioxy-N-ethylamphetamine (also known as N-ethyl-alpha-methyl-3,4-(methylenedioxy)phenethylamine, N-ethyl-MDA, MDE, MDEA 7404
	(9) N-hydroxy-3,4-methylenedioxyamphetamine (also known as N-hydroxy-alpha-methyl-3,4-(methylenedioxy)phenethylamine, and N-hydroxy-MDA 7402
(3) 3,4,5-trimethoxy amphetamine	(10) 3,4,5-trimethoxy amphetamine 7390
(4) Bufotenine	(11) Bufotenine 7433 Some trade and other names: 3-(beta-Dimethylaminoethyl)-5-hydroxyindole; 3-(2-dimethylaminoethyl)-5-indolol; N,N-dimethylserotonin; 5-hydroxy-N,N-dimethyltryptamine; mappine
(5) Diethyltryptamine	(12) Diethyltryptamine 7434 Some trade and other names: N,N-Diethyltryptamine; DET
(6) Dimethyltryptamine	(13) Dimethyltryptamine 7435 Some trade or other names: DMT
(8) Ibogaine	(14) Ibogaine 7260 Some trade and other names: 7-Ethyl-6,6-beta,7,8,9,10,12,13-octahydro-2-methoxy-6,9-methano-5H-pyrido [1', 2':1,2] azepino [5,4-b] indole; Tabernanthe iboga
(9) Lysergic acid diethylamide	(15) Lysergic acid diethylamide 7315
(10) Marihuana	(16) Marihuana 7360
(11) Mescaline	(17) Mescaline 7381

(18) Parahexyl 7374

Some trade or other names: 3-Hexyl-1-hydroxy-7,8,9,10-tetrahydro-6,6,9-trimethyl-6H-dibenzo[b,d]pyran; Synhexyl

(12) Peyote

(19) Peyote 7415

Meaning all parts of the plant presently classified botanically as *Lophophora williamsii Lemaire*, whether growing or not, the seeds thereof, any extract from any part of such plant, and every compound,, manufacture, salts, derivative, mixture or preparation of such plant, its seeds or extracts (Interprets 21 USC 812(c), Schedule I(c) (12)

(13) N-ethyl-3-piperidyl benzilate

(20) N-ethyl-3-piperidyl benzilate7482

(14) N-methyl-3-piperidyl benzylate

(21) N-methyl-3-piperidyl benzylate 7484

(15) Psilocybin

(22) Psilocybin 7437

(16) Psilocin

(23) Psilocin 7438

(17) Tetrahydrocannabinols

(24) Tetrahydrocannabinols 7370

Synthetic equivalents of the substances contained in the plant, or in the resinous extractives of *Cannibis* sp. and/or synthetic substances, derivatives, and their isomers with similar chemical structure and pharmacclogical activity such as the following

Δ1 cis or trans tetrahydrocannabinol, and their optical isomers

Δ6 cis or trans tetrahydrocannabinol, and their optical isomers

Δ3,4 cis or trans tetrahydrocannabinol, and its optical isomers

(Since nomenclature of these substances is not internationally standardized, compounds of these structures, regardless of numerical designation of atomic positions covered.)

(25) Ethylamine analog of phencyclidine 7455

Some trade or other names: N-ethyl-1-phenylcyclohexylamine, (1-phenylcyclohexyl)-ethylamine, N-(1-phenylcyclohexyl)ethylamine, cyclohexamine, PCE

(26) Pyrrolidine analog of phecyclidine 7458

Some trade or other names: 1-(1-phenylcyclohexyl)-pyrrolidine, PCPy, PHP

(27) Thiophene analog of phencyclidine 7470

Some trade or other names: 1-[1-(2-thienyl)-cyclohexyl]-piperidine, 2-thienyl analog of phencyclidine, TPCP, TCP

(28) 1-[1-(2-thienyl)cyclohexyl]-pyrrolidine 7473

(Some other names: TCPy)

(e) *Depressants.* Unless specifically excepted or unless listed in another schedule, any material, compound, mixture, or preparation which contains any quantity of the following substances having a depressant effect on the central nervous system, including its salts, isomers, and salts of isomers whenever the existence of such salts, isomers, and salts of isomers is possible within the specific chemical designation:

(1) Mecloqualone	2572
(2) Methaqualone	2565

(f) *Stimulants.* Unless specifically excepted, or unless listed in another schedule, any material, compound, mixture, or preparation which contains any quantity of the following substances having a stimulant effect on the central nervous system, including its salts, isomers, and salts of isomers:

(1) Fenethylline	1503
(2) (+/-) cis-4-methylaminorex ((+/-)cis-4,5-dihydro-4-methyl-5-phenyl-2-oxazolamine	1590
(3 N-ethylamphetamine	1475
(4) N,N-dimethylamphetamine (also known as N,N-trimethylbenzeneethanamine; N,N-alpha-trimethylphenethylamine	1480

(g) *Temporary listing of substances subject to emergency scheduling.* Any material, compound, mixture or preparation which contains any quantity of the following substances:

(1) N-[1-benzyl-4-piperidyl]-N-phenylpropanamide (benzylfentanyl), its optical isomers, salts, and salts of isomers	9818
(2) N-[1-(2-thienyl)methyl-4-piperidyl]-N-phenylpropanamide (thenylfentanyl), its optical isomers, salts, and salts of isomers	9834

Schedule II

§ 1308.12 Schedule II

(a) Schedule II shall consist of the drugs and other substances, by whatever official name, common or usual name, chemical name, or brand name designated, listed in this section. Each drug or substance has been assigned the Controlled Substances Code Number set forth opposite it.

(a) Unless specifically excepted or unless listed in another schedule, any of the following substances whether produced directly or indirectly by extraction from substances of vegetable origin, or independently by means of chemical synthesis, or by a combination of extraction and chemical synthesis:

(1) Opium and opiate, and any salt, compound, derivative, or preparation of opium or opiate.

(2) Any salt, compound, derivative, or preparation thereof which is chemically equivalent or identical with any of the substances referred to in clause (1), except that these substances shall not include the isoquinoline alkaloids of opium.

(3) Opium poppy and poppy straw.

(4) Coca leaves and any salt, compound, derivative, or preparation of coca leaves, and any salt, compound, derivative, or preparation thereof which is chemically equivalent or identical with any of these substances, except that the substances shall not include decocanized coca leaves or extraction of coca leaves, which extractions do not contain cocaine or ecgonine.

(b) Substances, vegatable origin or chemical systhesis. Unless specifically excepted or unless listed in another schedule, any of the following substances whether produced directly or indirectly by extraction from substances of vegetable origin, or independently by means of chemical synthesis, or by a combination of extraction and chemical synthesis:

(1) Opium and opiate, and any salt, compound, derivative, or preparation of opium, or opiate excluding apomorphine, dextrophan, nalbuphine, naloxone, and naltrexone, and their respective salts, but including the following:

(1) Raw opium	9600
(2) Opium extracts	9610
(3) Opium fluid	9620
(4) Powdered opium	9639
(5) Granulated opium	9640
(6) Tincture of opium	9630
(7) Codeine	9050
(8) Ethylmorphine	9190
(9) Etorphine hydrochloride	9059
(10) Hydrocodone	9193
(11) Hydromorphone	9150
(12) Metopon	9260
(13) Morphine	9300
(14) Oxycodone	9143
(15) Oxymorphone	9652
(16) Thebaine	9333

(2) Any salt, compound, derivative, or preparation thereof which is chemically equivalent or identical with any of the substances referred to in paragraph (b) (1) of this section, except that these substances shall not include the isoquinoline alkaloids of opium.

(3) Opium poppy and poppy straw.

(4) Coca leaves (9040) and any salt, compound, derivative, or preparation of coca leaves (including cocaine (9041) and ecgonine (9180) and their salts, isomers, derivatives and salts of isomers and derivatives), and any salt, compound, derivative, or preparation thereof which is chemically equivalent or identical with any of these substances, except that the substances shall not include decocanized coca leaves or extraction of coca leaves, which extractions do not contain cocaine or ecgonine.

(5) Concentrate of poppy straw (the crude extract of poppy straw in either liquid, solid, or powder form which contains the phenanthrene alkaloids of opium poppy), 9670

(b) Unless specifically excepted or unless listed in another schedule, any of the following opiates, including their isomers, esters, ethers, salts, and salts of isomers, esters and ethers, whenever the existence of such isomers, esters, ethers, and salts is possible within the specific chemical designation:

(1) Alphaprodine
(2) Anileridine
(3) Bezitramide
(4) Dihydrocodeine
(5) Diphenoxylate
(6) Fentanyl
(7) Isomethadone
(8) Levomethorphan
(9) Levorphanol
(10) Metazocine
(11) Methadone
(12) Methadone-Intermediate, 4-cyano-2-dimethylamino-4,4-diphenyl butane
(13) Moramide-Intermediate, 2-methyl-3-morpholino-1,1-diphenylpropane-carboxylic acid
(14) Pethidine
(15) Pethidine-Intermediate-A, 4-cyano-1-methyl-4-phenylpiperidine
(16) Pethidine-Intermediate-B, ethyl-4-phenylpiperidine-4-carboxylate
(17) Pethidine-Intermediate-C, 1-methyl-4-phenylpiperidine-4-carboxylic acid
(18) Phenazocine
(19) Piminodine
(20) Racemethorphan
(21) Racemorphan

(c) Unless specifically excepted or unless listed in another schedule, any injectable liquid which contains any quantity of methamphetamine, including its salts, isomers, and salts of isomers.

(c) *Opiates.* Unless specifically excepted or unless in another schedule, any of the following opiates, including their isomers, esters, ethers, salts, and salts of isomers, esters and ethers, whenever the existence of such isomers, esters, ethers, and salts is possible within the specific chemical designation, dextrorphan and levopropoxyphene excepted:

(1) Alphentanil 9737
(2) Alphaprodine 9010
(3) Anileridine 9020
(4) Bezitramide 9800
(5) Bulk dextropropoxyphene (non-dosage form) 9273
(6) Carfentanil 9743
(7) Dihydrocodeine 9120
(8) Diphenoxylate 9170
(9) Fentanyl 9801
(10) Isomethadone 9226
(11) Levomethorphan 9210
(12) Levorphanol 9220
(13) Metazocine 9240
(14) Methadone 9250
(15) Methadone-Intermediate, 4-cyano-2-dimethylamino-4,4-diphenyl butane 9254
(16) Moramide-Intermediate, 2-methyl-3-morpholino-1,1-diphenylpropane-carboxylic acid 9802
(17) Pethidine (meperidine) 9230
(18) Pethidine-Intermediate-A, 4-cyano-1-methyl-4-phenylpiperidine 9232
(19) Pethidine-Intermediate-B, ethyl-4-phenylpiperidine-4-carboxylate 9233
(20) Pethidine-Intermediate-C, 1-methyl-4-phenylpiperidine-4-carboxylic acid 9234
(21) Phenazocine 9715
(22) Piminodine 9730
(23) Racemethorphan 9732
(24) Racemorphan 9733
(25) Sufentanil 9740

(d) *Stimulants.* Unless specifically excepted or unless listed in another schedule, any material, compound, mixture, or preparation which contains any quantity of the following substances having a stimulant effect on the central nervous system:

(1) Amphetamine, its salts, optical isomers, and salts of its optical isomers 1100

(2) Methamphetamine, its salts, isomers, and salts of its isomers 1105

(3) Phenmetrazine and its salts 1631

(4) Methylphenidate 1724

(e) *Depressants.* Unless specifically excepted or unless listed in another schedule, any material, compound, mixture, or preparation which contains any quantity of the following substances having a depressant effect on the central nervous system, including its salts, isomers, and salts of isomers, whenever the existence of such salts, isomers and salts of isomers is possible within the specific chemical designation:

(1) Amobarbital 2125

(2) Glutethimide 2550

(3) Pentobarbital 2270

(4) Phencyclidine 7471

(5) Secobarbital 2315

(f) *Hallucinogenic substances*

(1) Dronabinol (synthetic) in sesame oil and encaspulated in a soft gelatin capsule in a U.S. Food and Drug Administration approved drug product 7369

(Some other names for dronabinol: (6aR-*trans*)-6a,7,8,10a-tetrahydro-6,6,9-trimethyl-3-pentyl-6H-dibenzo[b,d]pyran-1-ol, or (-)-delta-9-(trans)-tetrahydrocannabinol)

(2) Nabilone 7379

(Another name for nabilone: (±)-*trans*-3-(1,1-dimethylheptyl)-6,6a,7,8,10,10a-hexahydro-1-hydroxy-6,6-dimethyl-9H-dibenzo-[b,d]pyran-9-one)

(g) *Immediate precursors.* Unless specifically excepted or unless listed in another schedule, any material, compound, mixture, or preparation which contains any quantity of the following substance:

(1) Immediate precursor to amphetamine and methamphetamine:

(i) Phenylacetone 8501

Some trade or other names: phenyl-2-propanone, P2P, benzyl methyl ketone, methyl benzyl ketone;

(2) Immediate precursor to phencyclidine (PCP):

(i) 1-phenycyclohexylamine 7460
(ii) 1-piperidinocyclohexanecarbonitrile PCC 8603

Schedule III

§ 1308.13 Schedule III

(a) Schedule III shall consist of the drugs and other substances, by whatever official name, common or usual name, chemical name, or brand name designated, listed in this section. Each drug or substance has been assigned the DEA Controlled Substance Code Number set forth opposite it.

(a) Unless specifically excepted or unless listed in another schedule, any material, compound, mixture, or preparation which contains any quantity of the following substances having a stimulating effect on the central nervous system:

(1) Amphetamine, its salts, optical isomers, and salts of its optical isomers
(2) Phenmetrazine and its salts
(3) Any substance (except and injectable liquid) which contains any quantity of methamphetamine, including its salts, isomers, and salts of isomers.
(4) Methylphenidate

(b) *Stimulants.* Unless specifically excepted or unless listed in another schedule, any material, compound, mixture or preparation which contains any quantity of the following substances having a stimulant effect on the central nervous system , including its salts, isomers (whether optical, position, or geometric), and salts of such isomers whenever the existence of such salts of isomers is possible within the specific chemical designation:

(1) Those compounds, mixtures, or preparations in dosage unit form containing any stimulant substances listed in Schedule II which compounds, mixtures, or preparations were listed on August 25, 1971, as excepted compounds under § 308.32, and any other drug of the quantitative composition shown in that list for those drugs or which is the same except that it contains a lesser quantity of controlled substances 1405
(2) Benzphetamine 1228
(3) Chlorphentermine 1645
(4) Clortermine 1647
(5) Phendimetrazine 1615

(b) Unless specifically excepted or unless in another schedule, any material, compound, mixture, or preparation which contains any quantity of the following substances having a depressant effect on the central nervous system:

(c) *Depressants.* Unless specifically excepted or unless listed in another schedule, any material, compound, mixture, or preparation which contains any quantity of the following substances having a depressing effect on the central nervous system:

(1) Any compound, mixture or preparation containing:
(i) Amobarbital 2126
(ii) Secobarbital 2316
(iii) Pentobarbital 2271

(1) Any substance which contains any quantity of a derivative of barbituric acid, or any salt of a derivative of barbituric acid.
(2) Chorhexadol (sic)
(3) Glutethimide
(4) Lysergic acid
(5) Lysergic acid amide
(6) Methyprylon
(7) Phencyclidine
(8) Sulfondiethylmethane
(9) Sulfonethylmethane
(10) Sulfomethane

or any salt thereof and one or more other active medicinal ingerdients which are not listed in any schedule.

(2) Any suppository dosage form containing:

(i) Amobarbital	2126
(ii) Secobarbital	2316
(iii) Pentobarbital	2271

or any salt of any of these drugs and approved by the Food and Drug Administration for marketing only as a suppository.

(3) Any substance which contains any quantity of a derivative of barbituric acid or any salt thereof 2100

(4) Chlorhexadol 2510

(5) Lysergic acid 7300
(6) Lysergic acid amide 7310
(7) Methyprylon 2575

(8) Sulfondiethylmethane 2600
(9) Sulfonethylmethane 2605
(10) Sulfonmenthane 2610

(11) Tiletamine and zolazepam or any salt thereof 7295

Some trade or other names for a tiletamine zolazepam combination product: Telazol

Some trade or other names for tiletamine: 2-(ethylamino)-2-(2-thienyl)-cyclohexanone

Some trade or other names for zolazepam: 4-(2-fluorophenyl)-6,8-dihydro-1,3,8-trimethylpyrazolo-[3,4-e] [1,4]-diazepin-7(1H)-one, flupyrazapon

(c) Nalorphine

(d) Nalorphine 9400

(e) *Anabolic Steroids* The term 'anabolic steroid' means any drug or hormonal substance, chemically and pharmacologically related to testosterone (other than estrogens, progestins, and corticosteroids) that promotes muscle growth, and includes —

(i) boldenone	4115
(ii) 4-chlorotestosterone	4118
(iii) clostebol	4118
(iv) dehydrochloromethyltestosterone	4119
(v) dihydrotestosterone	4175
(vi) drostanolone	4121
(vii) ethylestrenol	4124
(viii) fluoxymesterone	4127
(ix) formebolone	4130

(x) mesterolone 4133
(xi) methandienone 4139
(xii) methandranone
(xiii) methandriol 4136
(xiv) methandrostenolone 4139
(xv) methenolone 4142
(xvi) methyltestosterone 4145
(xvii) mibolerone 4151
(xviii) nandrolone 4154
(xix) norethandrolone 4160
(xx) oxandrolone 4163
(xxi) oxymesterone 4166
(xxii) oxymetholone 4169
(xxiii) stanolone 4175
(xxiv) stanozolol 4179
(xxv) testolactone 4184
(xxvi) testosterone 4187
(xxvii) trenbolone 4190
(xxviii) any salt, ester, or isomer of a drug or substance described or listed in this paragraph, if that salt, ester, or isomer promotes muscle growth.

(d) Unless specifically excepted or unless listed in another schedule, any material, compound, mixture, or preparation containing limited quantities of any of the following narcotic drugs, or any salt thereof:

(1) Not more than 1.8 grams of codeine per 100 milliliters or not more than 90 milligrams per dosage unit, with an equal or greater quantity of an isoquinoline alkaloid of opium

(2) Not more than 1.8 grams of codeine per 100 milliliters or not more than 90 milligrams per dosage unit, with one or more active, nonnarcotic ingredients in recognized therapeutic amounts

(3) Not more than 300 milligrams of dihydrocodeinone per 100 milliliters or not more than 15 milligrams per dosage unit, with a four-fold or greater quantity of an isoquinoline alkaloid of opium

(4) Not more than 300 milligrams of dihydrocodeinone per 100 milliliters or not more than 15 milligrams per dosage unit, with one or more active, nonnarcotic ingredients in recognized therapeutic amounts

(5) Not more than 1.8 grams of dihydrocodeine per 100 milliliters or not more than 90 milligrams per dosage unit, with one or more active, nonnarcotic ingredients in recognized

(f) *Narcotic Drugs* Unless specifically excepted or unless listed in another schedule, any material, compound, mixture, or preparation containing any of the following narcotic drugs, or their salts calculated as the free anhydrous base or alkaloid, in limited quantities as set forth below:

(1) Not more than 1.8 grams of codeine per 100 milliliters or not more than 90 milligrams per dosage unit, with an equal or greater quantity of an isoquinoline alkaloid of opium 9803

(2) Not more than 1.8 grams of codeine per 100 milliliters or not more than 90 milligrams per dosage unit, with one or more active, nonnarcotic ingredients in recognized therapeutic amounts 9804

(3) Not more than 300 milligrams of dihydrocodeinone (hydrocodone) per 100 milliliters or not more than 15 milligrams per dosage unit, with a four-fold or greater quantity of an isoquinoline alkaloid of opium 9805

(4) Not more than 300 milligrams of dihydrocodeinone (hydrocodone) per 100 milliliters or not more than 15 milligrams per dosage unit, with one or more active, nonnarcotic ingredients in recognized therapeutic amounts 9806

(5) Not more than 1.8 grams of dihydrocodeine per 100 milliliters or not more than 90 milligrams per dosage unit, with one or more active, nonnarcotic ingredients in recognized

therapeutic amounts

(6) Not more than 300 milligrams of ethylmorphine per 100 milliliters or not more than 15 milligrams per dosage unit, with one or more active, nonnarcotic ingredients in recognized therapeutic amounts

(7) Not more than 500 milligrams of opium per 100 milliliters or per 100 grams, or not more than 25 milligrams per dosage unit, with one or more active, nonnarcotic ingredients in recognized therapeutic amounts

(8) Not more than 50 milligrams of morphine per 100 milliliters or per 100 grams with one or more active, nonnarcotic ingredients in recognized therapeutic amounts

Schedule IV

(1) Barbital

therapeutic amounts 9807

(6) Not more than 300 milligrams of ethylmorphine per 100 milliliters or not more than 15 milligrams per dosage unit, with one or more active, nonnarcotic ingredients in recognized therapeutic amounts 9808

(7) Not more than 500 milligrams of opium per 100 milliliters or per 100 grams, or not more than 25 milligrams per dosage unit, with one or more active, nonnarcotic ingredients in recognized therapeutic amounts 9809

(8) Not more than 50 milligrams of morphine per 100 milliliters or per 100 grams with one or more active, nonnarcotic ingredients in recognized therapeutic amounts 9810

§ 1308.14 Schedule IV

(a) Schedule IV shall consist of the drugs and other substances by whatever official name, common or usual name, or brand name designated, listed in this section. Each drug or substance has been assigned the DEA Controlled Substance Code Number set forth opposite it.

(b) *Narcotic drugs.* Unless specifically excepted or unless listed in another schedule, any material, compound, mixture, or preparation containing any of the following narcotic drugs, or their salts calculated as the free anhydrous base or alkaloid, in limited quantities as set forth below:

(1) Not more than 1 milligram of difenoxin and not less than 25 micrograms of atropine sulfate per dosage unit 9167

(2) Dextrop[r]oxyphene (alpha-(+)-4-dimethylamino-1,2-diphenyl-3-methyl-2-propionoxybutane) 9278

(c) *Depressants.* Unless specifically excepted or unless listed in another schedule, any material, compound, mixture, or preparation which contains any quantity of the following substances, including its salts, isomers, and salts of isomers, whenever the existence of such salts, isomers and salts of isomers is possible within the specific chemical designation:

(1) Alprazolam 2882
(2) Barbital 2145
(3) Bromazepam 2748
(4) Camazepam 2749

(2) Chloral betaine	(5) Chloral betaine	2460
(3) Chloral hydrate	(6) Chloral hydrate	2465
	(7) Chlordiazepoxide	2744
	(8) Clobazam	2751
	(9) Clonazepam	2737
	(10) Clorazepate	2768
	(11) Clotiazepam	2752
	(12) Cloxazolam	2753
	(13) Delorazepam	2754
	(14) Diazepam	2765
	(15) Estazolam	2756
(4) Ethchlorvynol	(16) Ethchlorvynol	2540
(5) Ethinamate	(17) Ethinamate	2545
	(18) Ethyl loflazepate	2758
	(19) Fludiazepam	2759
	(20) Flunitrazepam	2763
	(21) Flurazepam	2767
	(22) Halazepam	2762
	(23) Haloxazolam	2771
	(24) Ketazolam	2772
	(25) Loprazolam	2773
	(26) Lorazepam	2885
	(27) Lormetazepam	2774
	(28) Mebutamate	2800
	(29) Medazepam	2836
(7) Meprobamate	(30) Meprobamate	2820
(6) Methohexital	(31) Methohexital	2264
(8) Methylphenobarbital	(32) Methophenobarbital (mephobarbital)	2250
	(33) Midazolam	2884
	(34) Nimetazepam	2837
	(35) Nitrazepam	2834
	(36) Nordiazepam	2838
	(37) Oxazepam	2835
	(38) Oxazolam	2839
(9) Paraldehyde	(39) Paraldehyde	2585
(10) Petrichloral	(40) Petrichloral	2591
(11) Phenobarbital	(41) Phenobarbital	2285
	(42) Pinazepam	2883
	(43) Prazepam	2764
	(44) Quazepam	2881
	(45) Temazepam	2925
	(46) Tetrazepam	2886
	(47) Triazolam	2887

(d) *Fenfluramine.* Any material, compound, mixture, or preparation which contains any quantity of the following substances, including its salts, isomers (whether optical, positional, or geometric), and salts of such isomers, whenever the existence of salts, isomers, and salts of isomers is possible:

(1) Fenfluramine	1670

(e) *Stimulants.* Unless specifically excepted or unless listed in another schedule, any material, compound, mixture, or preparation which contains any quantity of the following substances having a stimulant effect on the central nervous system, including its salts, isomers, and salts of isomers:

(1) Cathine ((+)-norpseudoephedrine 1230
(2) Diethylpropion 6160
(3) Fencamfamin 1760
(4) Fenproporex 1575
(5) Mazindol 1605
(6) Mefenorex 1580
(7) Pemoline (including organometallic complexes and chelates thereof) 1530
(8) Phentermine 1640
(9) Pipradrol 1750
(10) SPA ((-)-1-dimethylamino-1,2-diphenylethane) 1635

(f) *Other substances.* Unless specifically excepted or unless listed in another schedule, any material, compound, mixture, or preparation which contains any quantity of the following substances, including its salts:

(1) Pentazocine 9709

Schedule V

§ 1308.15 Schedule V

(a) Schedule V shall consist of the drugs and other substances, by whatever official name, common or usual name, or brand name designated, listed in this section.

(b) *Narcotic drugs.* Unless specifically excepted or unless listed in another schedule, any material, compound, mixture, or preparation containing any of the following narcotic drugs and their salts as set forth below:

(1) Buprenorphine 9064

Any compound, mixture, or preparation containing any of the following limited quantities of narcotic drugs, which shall include one or more nonnarcotic active medicinal ingredients in sufficient proportion to confer upon the compound, mixture, or preparation valuable medicinal quali-

(c) Narcotic drugs containing nonnarcotic active medicinal ingredients. Any compound, mixture, or preparation containing any of the following narcotic drugs, or their salts calculated as the free anhydrous base or alkaloid, in limited quantities as set forth below, which shall include

ties other than those possessed by the narcotic drug alone:

(1) Not more than 200 milligrams of codeine per 100 milliliters or per 100 grams.

(2) Not more than 100 milligrams of dihydrocodeine per 100 milliliters or per 100 grams.

(3) Not more than 100 milligrams of ethylmorphine per 100 milliliters or per 100 grams.

(4) Not more than 2.5 milligrams of diphenoxylate and not less than 25 micrograms of atropine sulfate per dosage unit.

(5) Not more than 100 milligrams of opium per 100 milliliters or per 100 grams.

one or more nonnarcotic active medicinal ingredients in sufficient proportion to confer upon the compound, mixture, or preparation valuable medicinal qualities other than those possessed by narcotic drugs alone:

(1) Not more than 200 milligrams of codeine per 100 milliliters or per 100 grams.

(2) Not more than 100 milligrams of dihydrocodeine per 100 milliliters or per 100 grams

(3) Not more than 100 milligrams of ethylmorphine per 100 milliliters or per 100 grams.

(4) Not more than 2.5 milligrams of diphe noxylate and not less than 25 micrograms of atropine sulfate per dosage unit.

(5) Not more than 100 milligrams of opium per 100 milliliters or per 100 grams.

(6) Not more than 0.5 milligrams of difenoxin and not less than 25 micrograms of atropine sulfate per dosage unit.

(d) *Stimulants.* Unless specifically exempted or excluded or unless listed in another schedule, any material, compound, mixture, or preparation which contains any quantity of the following substances having a stimulant effect on the central nervous system, including its salts, isomers and salts of isomers:

(1) Propylhexedrine	8161
(2) Pyrovalerone	1485

THE HISTORY OF THE FEDERAL DRUG LAW

The history of man has been told with many different frameworks as guides. Psychoactive drugs can serve as one of these. Man has historically been, and still is, associated with the use of drugs that are able to modify his body or mind. Each person has inevitably found one or more drugs that he personally approves of, and believes that others are inappropriate. To him, involvement with the first group constitutes drug use, and with the second, drug abuse.

Records of use of some drugs have existed from pre-Christian times: there is the use of Opium (poppy seed eating in mid- Europe, 2500 BC), of Alcohol (breweries in Egypt 3500 BC) and of Marihuana (China, 2500 BC). The earliest use of others may be equally ancient but the origins are more difficult to document (Khat in the Near East, tobacco in the New World, Peyote and Psilocybe in North America, and many other natural plant toxins in dozens of independent cultures). The American obsession with drug use and abuse springs from a rich universal heritage of social approval and disapproval of drugs. In the United States, the rebellions against the British tax on tea (Daughters of Liberty, 1770; Boston Tea Party, 1773) quite literally led in sequence to the passage of the English Parliamentary Coercive Acts, the formation of the First Continental Congress, and to the War of Independence. All of the earliest efforts to regulate and limit the use of drugs were enforced through the fiscal device of taxation, an approach that had remained in use until the passage of the present Controlled Substance Act (1970).

The record of the laws concerning Alcohol can be used to give an interesting historical perspective to our present day efforts to contain drugs such as Heroin and Cocaine. The medical profession has long held that the overuse of Alcohol is a physical disease, but the many temperance societies (the first was formed in 1789, and there were over 6000 by 1833) held that any Alcohol use implied moral incompleteness. The spokesmen for medicine and morality agreed that restrictions were needed. Some local legal controls were instituted as early as 1845 and a move was made on the national scale in 1862 with the passage of the Internal Revenue Act (a tax on all retail liquor). The political picture reflected this consensus with the formation of the Prohibition Party in 1869 and the WCTU in 1874. Between 1880 and 1900, the inclusion of "temperance education" and "anti-alcohol teaching" was made a legal requirement in all states. Yet, during this period from 1870 to 1915 and the passage of the Sixteenth Amendment establishing the authority for the Federal income tax, between one half and two-thirds of all the internal revenue of the United States came from the taxation of alcohol. The culmination of this anti-alcohol hysteria was the passage of the Eighteenth Amendment (prohibition) in 1919 which was not repealed until 1933.

A similar story can be told with Cocaine, with Heroin, and with Marijuana, except that in these examples there has not yet been any move seriously entertained that would be parallel to the repeal of the laws that prohibited transactions related to Alcohol.

The background leading up to the passage of the Controlled Substances Act in 1970, and the changes in it, and amendments to it, are listed below chronologically. Several of the recent laws had been passed with some purpose other than narcotic law enforcement as an intended goal, but they are included since they have been found to be valuable when applied in this direction.

1886

One of the first laws of the United States against drug abuse prohibited the importation of Opium into the United States by any Subject of China (February 23, 1887). This act could be punished by from one to six months in prison or up to a $500 fine. Although Opium could be legally imported from Canada through the 1890's, there was concern over its importation by smuggling. When the duty was lowered from $12 to $6 a year, the smuggling stopped, and the volume of legal importation soared to 100,000 pounds per year.

1903

In August, 1903, there was established a U.S. Opium Commission to study the methods of regulation and control of Opium. During the years of this study, the use increased and according to the Federal Opium Commissioner, Hamilton Wright, some 160,000 pounds of Opium were imported for smoking and eating in 1907. And in 1906, 2,600,000 pounds of Coca leaves were imported. These abuses led to the Harrison Narcotics Act (1914-1915) which was the principal drug law of the United States for 55 years.

1906

The Pure Food and Drug Act was enacted, with the purpose of preventing the writing of false and misleading labels. This was not an anti-drug law but rather an indirect attempt to regulate drug distribution. The act was ineffective as it was essentially never enforced.

1909

Congress enacted the first legislation aimed at controlling drug abuse, the Act of 9 February, 1909, which specifically prohibited the importation of Opium for other than medicinal purposes. That year there was a Conference of the International Commission in Shanghai which recommended that all participating governments (thirteen, including the United States) take drastic measures to control the manufacture, sale and distribution of Morphine.

1912

The Hague Convention was held, and concluded that there was a need for the control of the domestic sale and use of Opium and of Coca.

1914

On December 17, 1914, President Wilson signed 26 U.S.C. 4701, the Federal Narcotics-Internal Revenue Regulations, commonly called the Harrison Narcotics Act. The effective date was March 1, 1915. This Act divided drugs into four classes: Class A contained drugs that were highly addictive, Class B contained drugs that were considered to possess little addiction liability. Classes X and M contained exempt and especially exempt narcotics, respectively. This bill was a tax measure, not a commerce measure, and was written to license and tax all who import (or manufacture, sell, or dispense) Opium or Cocaine (and their derivatives).

A companion act was passed the same year, Narcotic Drugs Import and Export Act. Neither of these bills received much comment at the time — the intense debates were concentrated on the prohibition movement.

It is interesting that at the time of the signing of this act, Cocaine was considered as a much more serious problem than either Heroin or the Opiates. At that time 46 of the 48 states had already adopted legislation regulating the distribution of Cocaine; only 29 had any legislation involving opiates.

A problem that developed at this time was the assignment of addicts (by physicians) as patients. The Act was amended (Act of February 24, 1919) to stop the maintenance of addicts by medical treatment. Thus the law enforcement community viewed users as criminals. Physicians were arrested and convicted for their stand on this matter.

1922

An amendment to the Narcotic Drugs Import and Export Act of 1914 banned Cocaine entirely, and placed severe restrictions on the importation of Coca leaves and Opium. This was the first definition of Cocaine as a "narcotic."

1929

On January 19, 1929, President Coolidge signed the Porter Bill which established two narcotics farms for the confinement and treatment of federal prisoners who were addicts. This was an acknowledgement of the controversy that existed between the physicians and the legal wording.of the Harrison Act. On May 25, 1935, the United States Narcotic Farm at Lexington, Ky. was dedicated, and it was designed to treat 1400 addicts (voluntary patients and convicted violators of Federal Law).

1930

On June 14, 1930, Congress established the Bureau of Narcotics, within the Department of Treasury. President Hoover appointed Harry J. Anslinger to head the Bureau on September 23, 1930. He initiated the policy of attacking the supply of narcotics at its source.

1933

An international treaty was ratified by the League of Nations, limiting the manufacture of narcotic drugs to legitimate needs. This was hailed as a means of ending narcotic addiction.

1937

On August 2, 1937, the Marihuana Tax of 1937 was passed. In the early 1930's, the years of the Depression, it became widely believed that Marihuana was addicting and that its use caused insanity. Anslinger spoke widely concerning its presumed role in crime and mental deterioration. It had not been considered in the Harrison Act, and there was felt to be the need of control. The 1937 Marihuana Tax Act was passed calling for up to a $20,000 fine and 20 years in jail if a person possessed marijuana without a legal fiscal transfer stamp in his possession. The enforcement of this law was mainly directed against immigrants from Mexico. All of these moves and acts associated with enforcement were Treasury Department actions, rather than actions of the Justice Department.

1938

Food, Drug, and Cosmetic Act was enacted, requiring that drug manufacturers provide directions for drug use and proof of safety. The act was amended in 1962 to require the demonstration of efficacy, as well.

1942

The Opium Poppy Control Act was passed on December 11, 1942. This law was, as had been the previous laws, a revenue action, as its enforcement was directed towards the possession of a required tax stamp. The production of Opium had been encouraged domestically as it was difficult to import from oversees under wartime conditions. This law was an attempt to interfere with any illicit production.

1946

The Narcotics Act of 1946 was passed on March 8, 1946, as a parallel to the above Poppy Control Act, and provided controls for the synthetic equivalents of Opium and its derivatives, and for other drugs such as Cocaine.

1951

The Durham-Humphrey Amendment to the Federal Food, Drug, and Cosmetic Act was passed by Congress, which required that drugs which cannot be safely used without medical supervision should only be dispensed by prescription. Both the Barbiturates and the Amphetamines were gathered within this amendment.

This year, the first of several amendments to both the Harrison Narcotics Act and the Narcotic Drugs Import and Export Act were enacted, increasing penalties. The Acts were amended to standardize penalties between them. Here are the first requirements for mandatory prison sentences (they still could be suspended, however, and probation could be given).

1954

A Congressional hearing was held to evaluate the needs of imposing Federal controls on the distribution of Amphetamines. No legislation resulted, but in the late 1950's the FDA began taking action against people who were diverting Amphetamine from legitimate channels.

1956

The Narcotic Control Act of 1956 was passed on July 18 of that year. It was felt that more severe penalties might work better. Not only were the prison terms and fines increased for the violation of the narcotics laws, but the minimum penalty limits became mandatory by the elimination of suspended sentences, probation, and parole.

1960

The Narcotics Manufacturing Act of 1960 (April 22) was yet another move made in the attempt to restrict and thus control the illicit use of drugs. Manufacturing licenses were required, and the Treasury Department could establish manufacturing quotas. This was a move that attempted to establish control of synthetic drugs in a manner parallel to the controls that had been directed towards the natural narcotics by the passage of the Import Export Act.

1961

The United States participated in the United Nations Conference directed towards the establishment of a single convention on Narcotic Drugs.

1962

A number of drug amendments were passed in 1962, but no action was taken on the attempts to include restrictions on Amphetamine. In this year, a Presidential commission recommended strong Federal efforts be made against the diversion, from legitimate trade channels, of Amphetamine and other non-narcotic drugs.

The Racketeer Influenced and Corrupt Organizations Act (RICO, 18 U.S.C. 1962, 1963) was enacted into law. The main purpose of RICO was the elimination of the infiltration of organized crime and racketeering into legitimate organizations operating in interstate commerce. There was no indication that this law had been specifically intended to address criminal activity such as drug trafficking.

On October 19, 1970, Congress modified the RICO law to insert criminal forfeiture provisions.

Although there is a rich history of civil forfeiture involved in proceedings against property which has been involved in some wrong, the act of criminal forfeiture involves seizure of property of a person convicted of a felony. It was a common punishment in historic England, but was specifically prohibited in 1790 by the first Congress of the United States. As a result, criminal forfeitures were unknown in the United States for 180 years. This amendment to the RICO statute, and the continuing criminal enterprise section of the Controlled Substances Act (see below, 1970) are the first inclusion of this penalty in American history.

This act has been recently applied to the Hells Angels, in connection with Methamphetamine traffic.

1963

On January 15, 1963, President Kennedy established an Advisory Commission on Narcotic and Drug Abuse with Judge E. Barrett Prettyman as chairman. The commission filed its report at the end of the year with 25 strong recommendations which prompted President Johnson (July 15, 1964) to direct that the full power of the Federal government be applied to finding a solution to the national problem of drug abuse. A major recommendation was the transfer of all drug enforcement efforts to the Department of Justice. This actually occured five years later.

1964

The Navajo Indians of the Southwest won their right to use Peyote. Their religious ceremonies thus are not in violation of the narcotics laws.

1965

Congressman Harris introduced the Drug Abuse Control Amendments of 1965 (H.R. 2). This was approved by Congress July 15, 1965, and established within the FDA, the Bureau of Drug Abuse Control (BDAC). It became effective February 1, 1966, under the direction of John H. Finlator.

The FDA accepted the responsibility for the enforcement of this new law, which was limited in scope to stimulants and depressants (the

laws that related to narcotics and Marihuana were still administered by the Bureau of Narcotics under the Department of Treasury). For the first time, the authority of the FDA was not restricted to interstate commerce in drugs, and its agents could now, in some cases, make arrests without warrants. Although the role of the FDA in this particular matter was related to criminal law enforcement rather than to medical issues, it still engaged itself in educational projects geared to public consumption. However, despite this reorganization, the use of drugs continued to climb, and three years later, this structure was changed again.

1968

A presidential order (President Johnson, April 8, 1968) dictated a plan to merge the Bureau of Drug Abuse Control (of the Food and Drug Administration, under the Department of Health Education and Welfare) with the Bureau of Narcotics (of the Treasury Department). The result of this merger was named the Bureau of Narcotics and Dangerous Drugs. This brought the enforcement problems associated with drug abuse and criminal activity under a single authority, and under the Department of Justice.

On October 24, a number of additional amendments, and modifications of amendments to the Harrison Act were enacted. These were largely directed to again increase the penalties for violations of Federal law..

1970

On October 27, 1970,The Harrison Act was effectively removed from the books with the passage of the Controlled Substances Act of 1970. Title II of this Act was entitled Comprehensive Drug Abuse Prevention and Control Act. The synthetic psychoactive drugs, old and new, are now subjected to the same regulations as the older natural drugs. This is Public Law 91-513, dated October 27, 1970. It is offered as an amendment to the Public Health Service Act (and other laws) as a provision for increased research into, and prevention of, drug abuse and drug dependence; to provide for treatment and rehabilitation of drug abusers and drug dependent persons; and to strengthen existing law enforcement authority in the field of drug abuse.

With this act, Congress effectively destroyed the Federal-State relationship that existed between the Harrison Act and the Uniform Narcotic Drug Act. To restore this, the Commissioners on Uniform State Laws drafted the Uniform Controlled Substances Act. This Act is structured on the Federal act (five Schedules, etc.) and requires persons involved in the manufacture, distribution, and dispensing of scheduled drugs to obtain a registration form from the state. Each state can, however, impose its own penalties for violation of these laws.

The (then) Bureau of Narcotics and Dangerous Drugs (BNDD) is the agency within the Justice Department delegated to implement the Act for the Attorney General, and its primary concern is diversion, distribution, and enforcement. There are several new provisions in the Controlled Substances Act. One is the shift from just sale or transfer of a drug, to its possession with intent to transfer it as also being a criminal act. Also, this law can be applied to acts that are committed outside the Unites States if they are performed knowingly or with the intention of eventually violating laws within the United States.

The Food and Drug Administration (FDA) is delegated to implement the Act with the concurrence of the National Institute of Drug Abuse (NIDA). The primary concern of the FDA is safe and effective medical use, whereas the primary role of the NIDA is scientific research.

Two aspects of this bill have received special attention through its enforcement in the last decade.

Continuing Criminal Enterprise (CCE). A person is defined as engaging in a continuing criminal enterprise if he is an organizer or supervisor of a group of five or more persons who have obtained substantial income through a series of violations of this Act. He is not only subject to more severe penalties, but he shall also forfeit both profits and properties associated with his felonious acts.

No-knock entry. Following the legal issuance of a search warrant, an authorized agent may enter, forcibly and without warning, if he feels

that evidence might be otherwise disposed of, or if the warrant has allowed him to take such action.

See comments under the RICO act above (1962) concerning the history of criminal forfeiture.

1971

An international drug control treaty was written, the Convention on Psychotropic Substances, 1971. The United States became a party to this treaty in 1980, q.v.

1972

Congress passed Public Law 92-225 (March 21, 1972), the Drug Abuse Office and Treatment Act, aimed at reducing the incidence of drug abuse in the United States within the shortest period of time.

This established three new things. (1) Within the Executive Office, there was formed a Special Action Office for Drug Abuse Prevention (SAODAP). (2) There was established a National Council for Drug Abuse Prevention which provided for the formation of a National Drug Abuse Training Center. (3) There would be establishment of a National Institute on Drug Abuse (to be effective in December 31, 1974) with the eventual dissolution of SAODAP six months later.

It also authorized $1 billion to coordinate and expand Federal, State and private programs directed against drug use.

1973

The DEA was created. This organization was a merger of ODALE (the Office of Drug Abuse Law Inforcement, created in the Justice Department in 1972), of ONNI (the Office of National Narcotics Intelligence, also created in 1972 in the Justice Department), of the drug enforcement and intelligence functions of the U.S. Customs Service, and of the BNDD (the Bureau of Narcotics and Dangerous Drugs, which was itself a merger of BDAC —(the Bureau of Drug Abuse Control of the FDA under the Department of HEW, — and the Bureau of Narcotics, under the Department of the Treasury). The DEA is under the Department of Justice.

1974

On May 14, 1974, President Nixon signed Pub. Law 93-281, the Narcotic Addict Treatment Act of 1974, which had the purpose of amending the Controlled Substance Act of 1970 to provide for the registration of practitioners conducting narcotic maintenance and detoxification treatment programs for addicts.

1976

The House of Representatives established the Select Committee on Narcotics Abuse and Control to serve a review and advice function over the DEA. Other committees function in this area (the House and Senate Judiciary Committees, and the Senate's Permanent Subcommittee on Investigations) and finally, in 1981, the Senate Drug Enforcement Caucus was formed, to support legislation wanted by the drug law enforcement community. The DEA has provided the primary impetus for most of the following laws and amendments.

1978

On November 10, 1978, Congress passed the Psychotropic Substances Act of 1978 (Public Law 95-633). This is an amendment to the Comprehensive Drug Abuse Prevention and Control Act of 1970, and it allows the International Convention on Psychotropic Substances to suggest additions to the Act. Approval from Congress for this treaty had been sought since 1971. (The United States became a party to this treaty on April 15, 1980, q.v.). In addition, increased penalties and restrictions in the PCP area were included, including the placement of Piperidine in a position of having to be reported when possibly associated with Phencyclidine manufacture.

The very last section of this amendment is entitled as "Forfeiture of Proceeds of Illegal Drug Transactions." This addition states, quite simply, that all proceeds from drug transactions may be seized as forfeiture. This has given the government immediate possession of boats, airplanes, real property, and bank accounts, and has effectively limited the defense capabilities of a number of defendants. This asset seizure and forfeiture (before conviction) has

been felt by many law enforcement groups to have proven itself as an effective weapon in the area of drug use prevention.

1980

The United States became a party to the International Convention on Psychotropic Substances, 1971, effective April 15, 1980. Reference to the Psychotropic Substances Control Act of 1978 will give the groundwork behind this move. All drugs that are recommended for International control, are considered for scheduling under the CSA of the United States. At the time of the United State ratification of this treaty, there were drugs listed therein which were not recognized by U.S. law. The drug Parahexyl is an example, and reference to 47 FR 33986 is illustrative of the processes that have been set in motion.

1981

The Public Law 97-86 was enacted, entitled the "Department of Defense Authorization Act of 1982." This law includes a provision to revise the long-standing Posse Comitatus statute. Prior to this law, all military involvement in civil law enforcement was prohibited unless authorized by the Constitution or by some specific action of Congress. With this law, there was a clarification of the role of the military in civilian enforcement activities, and the assistance and support services which may be rendered by the military to law enforcement officials are defined.

There was quick implementation in the formation of the President's Task Force South Florida in January, 1982. This operation was geared to the interdiction of narcotics being smuggled into Florida from the Caribbean and from Latin America. The military aid provided included complex logistic and vessel support, aviation and radar surveillance, and the loan of equipment and facilities.

1982

On January 21, 1982, Attorney General William French Smith announced his decision to give the Federal Bureau of Investigation and the Drug Enforcement Administration "concurrent jurisdiction" over violations of the Federal drug laws. The general supervision of this joint effort was put in the charge of Francis M. Mullen, Jr. The priorities of the relationship were defined by the AG's specific order that the DEA will report to the FBI.

On September 2, 1982, there was enacted Public Law 97-248, the "Tax Equity and Fiscal Responsibility Act of 1982." Prior to this date the laws in effect (most recently, the Tax Reform Act of 1976) severely restricted disclosure of tax information. Complex financial transactions are often associated with sophisticated criminal activity, and this information was not available to Federal enforcement authorities. This new law included several provisions sought by the Justice Department "to facitiate the appropriate disclosure of tax information to Federal law enforcement agencies for criminal investigative purposes while maintaining safeguards needed to protect the privacy of innocent citizens."

Federal officials may now gain access to tax information which is a most valuable source of financial data necessary to prosecute narcotics trafficking and organized crime.

1983

The Comprehensive Crime Act of 1983 was presented to congress in its 98th session, as Senate S-829 and HR 2151. It carried some 42 reforms sought by the Department of Justice, involving bail, sentencing, civil forfeiture, the exclusionary rule, and drug regulation. This was passed as public law 98-743 below.

1984

The Comprehensive Crime Control Act of 1984 (Pub. L. 98-473) was passed as an amendment to section 201 of the CSA (21 U.S.C. 812) on October 12, 1984. A House Report (98-835) that accompanied this law states (according to the Fed. Reg. Vol. 50 No.132, July 10, 1985) states that:

"This new procedure (emergency scheduling) is intended by the committee to apply to what has been called "designer drugs," new chemical analogs or variations of existing controlled substances, or other new substances which have a psychedelic, stimulant or depressant

effect and have high potential for abuse."

Chapter III of this law is entitled "Comprehensive Forfeiture Act of 1984." This portion extends the boundaries of seizure and forfeiture to include both terms "civil" and "criminal." This can allow seizure of profits derived from criminal acts as well as of property associated with the actual commission of such acts.

Chapter V of this law is in two parts: Part A (Controlled Substances Penalties Amendments Act of 1984) establishes weights and penalties within the law. Part B (Dangerous Drug Diversion Control Act of 1984) establishes or extends definitions of terms used in the law (such as isomer and narcotic drug), and allows the temporary placement of a substance into Schedule I, without the usual requirements, if this act is needed to avoid an imminent hazard to the public safety. In any given appication, this emergency scheduling will expire in one year unless extended for up to six months.

For the regulations established for the enforcement of this law, see 51 FR 5370.
A $1.7 billion anti-drug bill was passed by

1986

On April 8, 1986, the President Reagan signed a National Security Decision Directive on April 8, 1986, stating that drug trafficking constituted a threat to the national security of the United States. This authorized the military to participate in all international law-enforcement activity that was drug-related, except for making seizures and arrests. These latter two restrictions were removed by an opinion published by the Justice Department on November 3, 1989.

Congress on October 17, 1986, and signed into law by President Reagan. This is the largest-ever financial commitment directed towards the drug problem. From the enforcement point of view it provides funds to Federal, State, and Local Law Enforcement groups, to the Coast Guard, to the Customs Service, for additional U.S. attorneys, marshals, narcotics agents, and Federal prisons. Penalties are increased for nearly all convictions.

An amendment to the Controlled Substances Act was part of this, entitled The Designer Drug Enforcement Act of 1986 or The Scheduled Drug Analog Bill, originally introduced in 1985 as HR 2977. Although inspired by the appearance of several synthetic analogs of Fentanyl, in its final form it dictates that the laws that had been specifically written to apply just to Controlled substances, shall now apply to any analog of these substances that is intended for human consumption. Exception is made only for drugs that have received FDA approval or exemption, so that in effect all human research work with drugs that are thought to resemble known stimulant, depressant, or hallucinogenic drugs must be cleared through the FDA. Analogs are defined as chemicals that possess a structure that is substantially similar to that of a Schedule I or II drug, or have stimulant, depressant, or hallucinogenic properties, or be promoted for human use as having these properties.

1988

The Anti-Drug Abuse Act of 1988, PL 100-690, was signed into law on November 18, 1988. It was directed towards the prevention of the manufacture of scheduled drugs, and towards the increasing of penalties to discourage their use.

The Controlled Substance Act was amended to include a listing of precursor chemicals, and a listing of essential chemicals. A requirement for record-keeping has been imposed on all transactions involviong these materials, in excess of threshold amounts to be determined by the Attorney General.

There was the authorization made for the amending of the sentencing guidelines and for the impostion of the death penalty if a homocide is commited in the course of a drug transaction. Civil penalties in conjunction with drug-related convictions now include entries such as the withdrawal of Federal benefits, the cancellation of FHA mortgage and of student loans, and the suspension of drivers' licenses.

Two statements of Congressional policy have been made. That the Congress finds, "That the legalization of illegal drugs, on the Federal or State level, is an unconscionable surrender in a

war in which [—] there can be no substitute for total victory." And that, "It is the declared policy of the United States Government to create a drug-free America by 1995.

1990

On November 29, 1990, President Bush signed the Crime Control Act of 1990, PL 101-647. It dealt with a number of drug-related matters such as international money laundering, asset forfeiture, and a general strengthening of penalties associated with drug crimes. There were several specific changes made, however, to the drug law itself.

The Anabolic Steroids Control Act of 1990 made an explicit addition of 27 steroids to the Schedule III group of controlled drugs. The listing also includes esters, which brings most of the more frequently used forms also under control. Steroids used in live stock are exempted, as are estrogens, progestins, and corticosteroids.

The Federal Food, Drug, and Cosmetic act has been amended to make the distribution of human growth hormone, outside of the use in disease or other recognized medical conditions, a felony violation of the Controlled Substances Act. The Drug Enforcement Administration is authorized to investigate offences involving somatrem, somatropin, or any related analogue of human growth hormone.

Some twelve new chemicals have been added to the list of "Precursor Chemicals." One of these, D-lysergic acid (an essential precursor to the manufacture of LSD) may present a procedural problem as it had already been listed as a Schedule III depressant.

The appearance of "ice", or smokable methamphetamine, as prompted the amendment of the existing sentencing guidelines to increase by two levels the penalty exacted if that crime involved smokable methamphetamine. As there is no suggestion as to how methamphetamine hydrochloride can be assigned a smokable, as opposed to a non-smokable, role, the increased penalties will probably be applied to all methamphetamine convictions.

One section addresses the question of inhalable alkyl nitrites. Unless for a purpose approved by the Food, Drug, and Cosmetic Act, all alkyl nitrites that may be used for inhaling or otherwise being employed for "euphoric or physical" effects, have been made unlawful. The Consumer Product Safety Act is now the authority for controlling these banned hazardous products.

Forensic Toxicology
BEHS 183 Lect. #27

THE EXPERT WITNESS AND COURT TESTIMONY

Those of you who are going into the area of criminalistics will with absolute certainty have your opportunity to be seated in the witness box, under oath, and be expected to give forth with fact and wisdom. Others may also have that chance, as they develop expertise in a technical area and might be called upon for opinions.

This is a unique environment, loaded with protocol and parliamentary maneuvering. There can be a vast gap between what is believed, what is said, and what is meant. There is no more valuable instructor than the court itself – attend a number of sessions when there is testimony being given by an experienced expert witness. Watch the procedure, the timing, the manner of expression.

THE COURT AND ITS VOCABULARY:

Witness:

A person who is called to give testimony concerning information wanted by one side, the other side, or the court itself, to help arrive at a decision in a trial. If the information deals with details of fact (what color was the car?) then the witness is a factual witness. If the information calls for an opinion (was the car driving too fast?) then the witness is an expert witness.

A factual witness is usually someone who was physically located or involved in the area of an alleged offense. He may be asked for as much detail as can be remembered, that was directly impinging upon him. Things seen in person, conversations involved in, in person, but nothing between others. What he said he heard, is hearsay, and not allowed. Also, almost always factual witnesses are sequestered, which means that while one witness is recalling his memories, the others are excluded from the court. You may be subpoenaed as a factual witness, and you must go.

An expert witness is quite a different matter. You rarely have been directly associated with the case ahead of time, but rather, you are being brought in specifically for an opinion. You have every right to employ hearsay in that this can be a most important source of information (I was talking to one of my colleagues about this at the hospital just the other day, and he said –). There rarely is sequestering, as you can only improve your understanding and arrive at a more precise opinion by hearing the testimony of others. You will expect to be paid for your time and expertise, and so you are rarely subpoenaed. But remember, that if your opinions have any value, they will be charged for.

Prosecution:

This is the hurt party, usually in criminal cases the people who have been offended by having one of their laws broken. In civil cases, this position is called that of the plaintiff. The damaged side is bringing charges and demanding redress.

Defense:

The person accused of doing the damage, and who is present with the assumption of innocence.

Voir-dire:

To see, to say. This is a ritual that confronts every expert witness before there is any examination whatsoever. It is an interview conducted on qualifications and on background but no specific aspects of the current case are touched upon. After questions from both sides, the friendly attorney (the one who called the witness in, in the first place) asks the court to accept this person as an expert in this area. Acceptance is almost almost automatic.

Direct examination:

The initial questioning of you by your attorney. This is usually friendly (the lawyer is paying you for your time, after all) and follows lines which the two of you have worked out before. It usually ends up with you being asked for your conclusions or opinions.

Cross examination:

Here can start the traumatic part of questions and answers. At the end of this lecture I mention several of the more common tactics employed, but the general rules are simple: Answer each question with care, with honesty, and not too quickly. Volunteer nothing beyond your answer. And both your attorney and the judge are your friends if something feels incorrect or uncomfortable.

Court reporter:

This is the inconspicuous person in the front of the judge's bench, who is tapping away on a small machine with a long roll of paper emerging from it. He is the only court record, he can record only one voice at a time, he can run out of paper. He is the only person other than the judge who can bring the whole process to a stop. And will, without hesitation, if there is a shouting match. He "cannot hear," and thus does not record, discussions at the bench.

Deposition:

The giving of a deposition is an unusually trying situation. This is testimony

that is gathered before the trial, and can be entered as a unit at the trial to save court time. You are with your friendly attorney, the other attorney, and a court reporter, but there is no judge, and no restraint as to where the questions might lead. Simply remember, that between the deposition and the trial you can learn something new and change your mind.

Interrogatory:

Often a written series of questions may be given you allowing the leisurely rearching out of answers, and the entire question-answer result (the interrogatory) can be admitted as testimony in trial. The purpose is to save the court's time, but any aspect of your answer that is felt should be challenged may be revived in court. Remember, in the time between an interrogatory, or a deposition, you can do additional research and, coming to a new and different conclusion, change your opinions.

CONDUCT ON THE STAND:

Your dress and appearance is important, especially to a jury. Think out, ahead of time, the body language. The jurors won't know why, but they will trust a witness more who has his hand away from his mouth and who doesn't cross his legs.

Resist all temptation to be humorous or to exploit a play on words.

During cross examination, listen to the exact phrasing of each question, as there may be some long-term plan on the part of the "unfriendly" lawyer to try for some new leads in your answers to questions. If you understand the question, then wait a moment, then answer clearly and as concisely as possible. If you wait too long, it can sound like "searching for a way to evade the question." If you answer too quickly, you might get ahead of your own attorney's "I object, your honor." And even if the question may be objectionable, the presence of the answer invalidates any subsequent ruling by the court.

With the court's permission, you may use notes, but reading from them weakens the effect of your testimony with the jury. Remember that the jury is a group of simple, inexperienced people of average intelligence, and has been carefully chosen that way by a tedious process of selection that preceded your ever being in court. You must establish eye-contact with someone in the jury (your attorney may suggest the most alert one there) and talk to him/her. That person may well be your spokesman in the jury room during deliberation.

Don't discuss anything with your attorney during recess (off the stand) in the presence of the jury. It will look like some sort of strategy plotting. Wait until they have retired.

You can get over the initial stage fright by being able to predict the first two questions exactly. They will be: "Do you solemnly swear or affirm etc. etc. – " to which you had better answer, "I do." And number 2: "Please state your full name and spell

your last name." If you blow that one, give up immediately.

As stated before, never volunteer anything that lies outside the question; it can give new ideas of territories yet unimagined to the other attorney.

PROBLEM QUESTIONS ON THE STAND

Here are some problems, pitfalls, and petty maneuvers from the other attorney on cross-examination, or on voir-dire:

Q. "Do you expect to get paid for your testimony?" The implication to the jury is that you will tailor your opinion for money.

A. "Of course." And if the next question is aimed at finding out how much, say that you have no idea, in that you have no way of estimating the time that you will have to invest. That will usually end that trick. It is aimed at inexperienced witnesses.

Q. "Can evidence for morphine usage always be found in urine?" This is called a leading question, in that the conclusion is to be found in the question, and your yes or no answer should be qualified. In general leading questions are not allowed on direct examination, but are often OK on cross examination, as a way of restating the earlier testimony to save time. Be careful. Wait for a friendly objection. If none, answer the question with the needed qualifications.

A. "The answer to that depend on many factors – –."

Q. "Do you believe that cocaine is a narcotic?" A demand for a yes or no answer to something that needs an explanation. Such a question is usually geared to the ears of the jury. Start your answer with the beginning of a qualification, and if there is an abrupt demand for a yes or no, and your attorney doesn't help, turn to the judge. He will allow you to answer in your own words.

A. "There is a legal definition, and a pharmacological definition, and a social definition for the term narcotic." Etc.

There are many devices used on cross examination, that are intended to confuse, anger, or trip up the witness. They are usually the last retreat of an attorney who sees his case falling apart. Be patient with such things as:

The rapid-fire question. Take your time.

The repetitious question. "I have just answered that."

The question in the form of a statement. "What was the question?"

Mispronunciation of your name, badgering you, being condescending, staring at you after you have completed your answer – all are easily handled if you simply

wait for the next question.

DISCLAIMERS AND AMNESIA:

There are two powerful allies that you must call upon when needed. One is the disclaimer, and the other is a failing memory. Both can be used to great advantage. Disclaimers are words such as:

Approximately
About
Roughly
Give-or-take
Similar to
Resembling

allow a concordance between your testimony and that of another witness, if the exacts ot quite the same. There will always be conflicting answers from different people, ıese disclaimers will keep everyone honest.

And to questions that might be hard to back up – I don't remember.

Q. "Did you come to the conclusion that the infra-red spectrum was indeed that of heroin BEFORE or AFTER you hear that Dr. Jones had come to that very conclusion?"

A. "I really don't remember. But that conclusion was inescapable from the spectrum that I obtained."

Expert testimony is a game; it is a dance that is constructed of many strange steps. It can only be learned by experience.