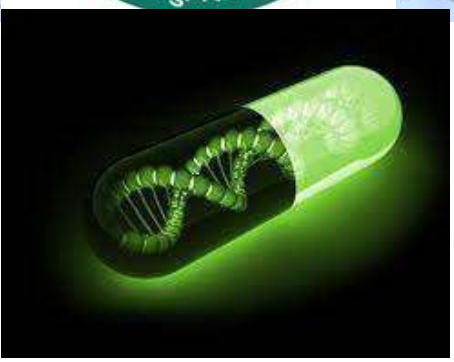
Department of Biochemistry

Newsletter

All India Institute of Medical Sciences,Rajkot



CLINIAL BIOCHEMISTRY & MOLECULAR BIOLOGY BULLETIN

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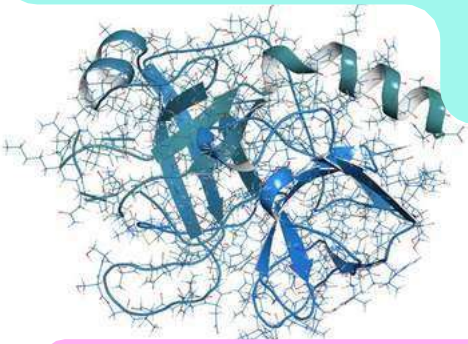
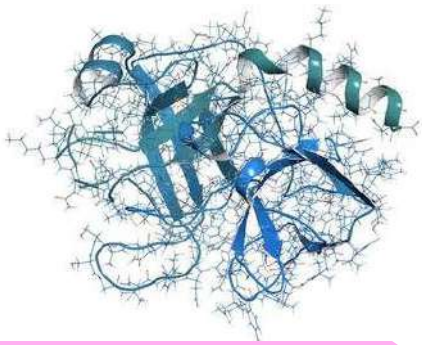
This issue is dedicated to

Topoisomerase II Poisons & The Dual Role They Play





Type 2 Topoisomerase Poisons: Converting Essential Enzymes into Molecular Scissors

DNA topoisomerases are enzymes that catalyze changes in the topological state of DNA. During DNA replication and transcription, there is overwinding of the DNA duplex.If left unchanged, this torsion would eventually stop the DNA or RNA polymerases. DNA topoisomerases corrects supercoils by binding to DNA and cutting the sugar-phosphate backbone of either one (type I topoisomerases) or both (type II topoisomerases) of the DNA strands.

This transient break allows the DNA to be untangled and then the nick is resealed. Type I & type II topoisomerase have further subtypes such as IA, IB, IC, IIA & IIB. Because of their crucial role in DNA replication, drugs targeted against them are developed for antibacterial and anticancer therapy.

Topoisomerase II poisons as antibacterial and anticancer agents.

Fluoroquinolones such as ciprofloxacin, levofloxacin, etc are very effective antibacterial agents that works by inhibiting DNA gyrase – a type of topoisomerase II. Aminocoumarins such as novobiocin, clorobiocin and coumermycin A1, are natural products from Streptomyces that inhibit the ATPase reaction of gyrase. Etoposide targets topoisomerase II by stabilizing the covalent cleavage complex and preventing re-ligation of the cleaved DNA. Doxorubicin and the related derivatives daunorubicin, epirubicin, and idarubicin also target topoisomerase II and stabilize cleavage complex. These agents are used as an anticancer drugs for the treatment of breast cancer, small-cell lung cancer, lymphoma and leukemias.

Dietary Topoisomerase II Poisons.

Topoisomerase II poisons are regularly consumed as part of the human diet. These include bioflavonoids (flavones, isoflavones, and flavonols), catechins, catechols, isothiocyanates, and quinones.

Bioflavonoids are a diverse group of polyphenolic compounds that are constituents of many fruits, vegetables, legumes, and plant leaves. These compounds can help protect against cancer, cardiovascular disease, osteoporosis, age-related diseases, and inflammation by acting on topoisomerase II. Catechins are commonly found in green tea, reduced risk of breast, prostate, colorectal, and lung cancer by inhibiting activity of topoisomerase II. Olive plants are a rich source of catechols such as Hydroxytyrosol, oleuropein, and verbascoside which work as covalent poison for topoisomerase II and give anticancer property to olive. Curcumin is the principal flavor and color component of the spice turmeric. In aqueous solution at physiological pH, it undergoes a spontaneous and complex autoxidation reaction that generates a series of quinone methide intermediates that work as covalent poison for topoisomerase II and give antibacterial & anticancer property. Thymoquinone of black seed also possess similar properties.

Environmental Topoisomerase II Poisons.

A variety of environmental quinone-based compounds that are damaging to human health are also potent topoisomerase II poisons. 1,4-benzoquinone, a major metabolite of benzene, is associated with the development of leukemia. Quinone metabolites of polychlorinated biphenyls (PCBs), which are used as industrial diluents, lubricants, and cooling fluids are highly carcinogenic. N-acetyl-p-benzoquinone imine (NAPQI), which is the toxic metabolite of acetaminophen, is known to be a potent liver toxin. 1,2-naphthoquinone, a secondary metabolite of naphthalene, is an environmental pollutant found in diesel exhaust.

In order to carry out their essential nuclear functions, type II topoisomerases generate transient double-stranded breaks in the genetic material. Consequently, although they are required for cell survival, type II topoisomerases are intrinsically dangerous enzymes. Topoisomerase II poisons exploit this hazardous property and convert the type II enzyme into "molecular scissors" that fragment the genome. Topoisomerase II poisons are structurally and mechanistically diverse, and the only apparent feature that some of them have in common is the ability to increase levels of topoisomerase II-mediated DNA cleavage complexes.

Topoisomerase II Catalytic Inhibitors vs. Poisons.

Compounds that alter topoisomerase II activity can be divided into two categories: catalytic inhibitors and poisons Topoisomerase II catalytic inhibitors are compounds that impair the overall catalytic activity without increasing the concentration of cleavage complexes.

Interfacial Poisons	Covalent Poisons
Act non-covalently at the active site and block ligation	Covalently adduct topoisomerase II at sites distal to the catalytic core
Enhance DNA cleavage when incubated with the enzyme prior to the addition of DNA	Inhibit topoisomerase II when incubated with the enzyme prior to the addition of DNA
Unaffected by reducing agents	Activity is blocked by reducing agents
(Examples: etoposide, doxorubicin, genistein, etc.)	(Examples: curcumin, EGCG, benzoquinone, etc.)

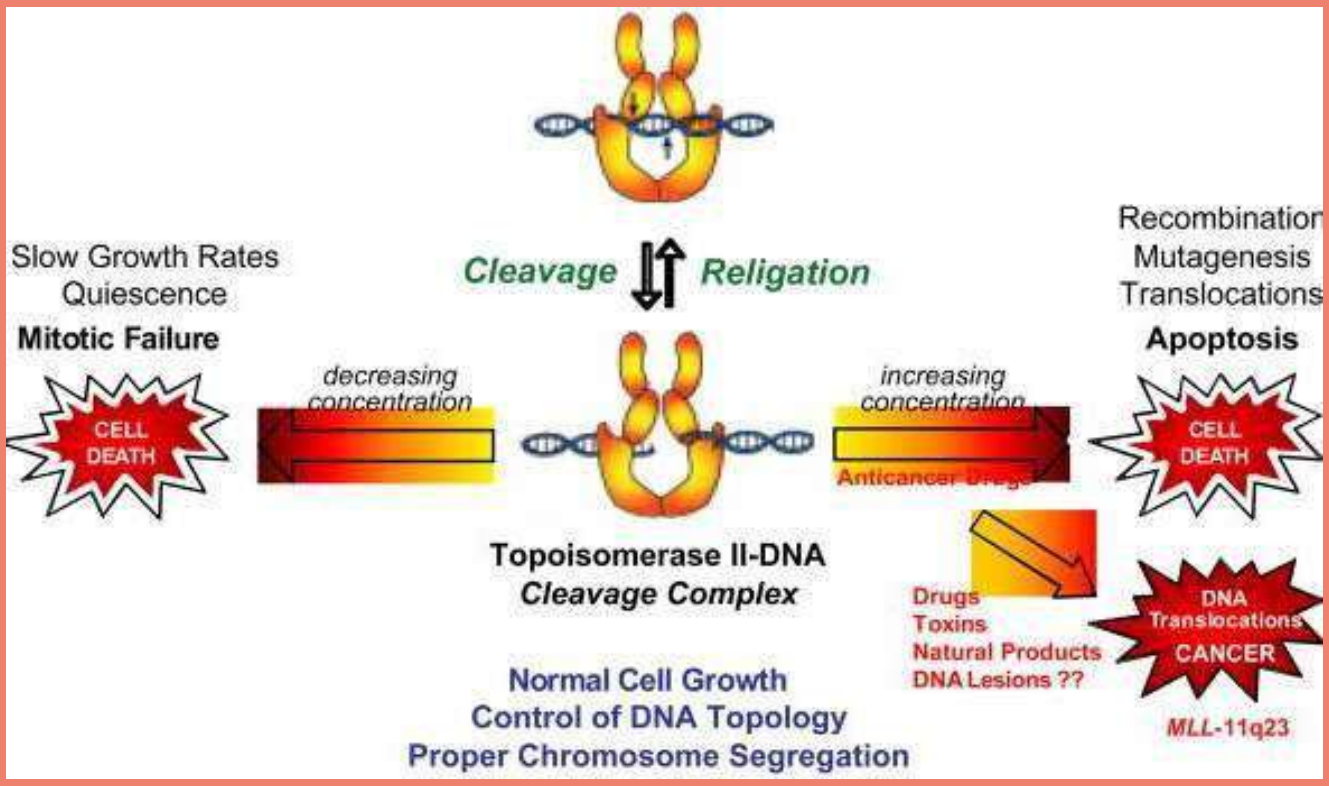


Image Courtsey: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2679583/

Experts Talk

Dr. Sanjay Gupta
Professor & Head
Department of Forensic Medicine & Toxicology

Laboratory diagnosis in cases of poisoning is not much explored in our country though they play a crucial role in diagnosis and treatment. There are only handful of tests which are usually performed and some of them just play a role in corelation and not diagnostics. Analytical toxicology is usually under the purview of forensic scientists. For which a sample needs to be properly collected by the attending doctor, judicious use of preservative, proper labelling, sealing and forwarding to forensic science laboratory. This takes a lot of time in getting the result of the test, sometime weeks and months. In cases of poisoning, some enzymes play a complex role and test results fall negative. In my practice, I have observed that there were definitive signs of poisoning although chemical analysis failed to detect the poison from the samples.

Analytical techniques like Thin Layer Chromatography (TLC) and Gas Liquid Chromatography with or without Mass Spectrometry or Spectrophotometry are found useful in quantitative and qualitative analysis of poison whether solid, liquid or gaseous. In cases of poisoning, Section 201 of Indian Penal Code (IPC) emphasized importance of maintaining chain of custody, proper preservation of evidences. Section 39 of CrPC (Criminal Procedural Code) emphasized and bound the doctor about relevance of divulging information to law enforcement agencies in public interest.

There are many reasons documented, which are responsible for modifying action of poisons like quantity of a poison, whether a poison is water or lipid soluble, route of administration, condition of the body, age of the person, habit, tolerance abilities, drug interaction, state of health, hypersensitivity, idiosyncrasy and genetic variation – G6PD deficiency etc.

Certain poisons including inebriant, stupefying, deliriant, narcotic and psychotropic substances, inorganic irritants, asphyxiants and food poisons are creating difficulties in laboratory diagnosis due to their complex mechanism of action.

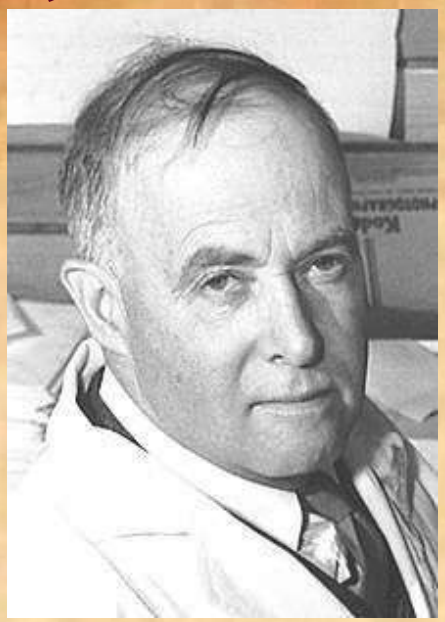
Laboratory diagnosis play key role in poisoning cases both in living and dead. There are only few certified poison information or detection centre. Topic chosen for this newsletter is very pertinent and relevant looking into the need of society and social justice.

Discovery of novel TOP2 catalytic inhibitors

Poison inhibitor of DNA Topoisomerase II (TOP2) are therapeutically employed as anti-cancer medication. They are known to have substantial adverse effects, including secondary malignancy and cardiotoxicity.
TOP2 catalytic inhibitors, on the other hand, cause minimal DNA damage, have low cytotoxicity, and effectively decrease the proliferation of cancer cells. They've been studied as possible cancer treatments.
New druggable pocket in TOP2 protein as its DNA binding protein has been identified from ZINC15 by virtual screening.
A catalytic TOP2 inhibitor, T60, was discovered to limit DNA cleavage by binding to and disrupting the DNA-protein interaction of TOP2. T60 is found to play a crucial role in inhibition of xenograft growth and tumor cell proliferation, despite of its minimal cytotoxicity. Furthermore, T60 also inhibits prostate cancer cell proliferation and activates androgen receptor. Hence, T60 appears to be a good approach for the development of novel therapeutic drug.
Compounds that can bind to DNA and prevent topoisomerase binding, such as the compounds aclarubicin and suramin, present another potential inhibitory mechanism, though specificity is again an issue with agents such as these.
Agents such as the compound merbarone, that can bind to the DNA-protein complex and prevent cleavage, represent yet another mechanism of catalytic inhibition.
Lastly, the ATP-dependent type II topoisomerases can be inhibited by agents that prevent ATP hydrolysis and DNA release after strand passage, as exemplified by the bisdioxopiperazine agent dexrazoxane. These agents result in a "closed clamp complex" that is analogous to the cleavage complex generated by the topoisomerase poisons.

Tid-Bits From History

James Batcheller Sumner: Father of Enzymology

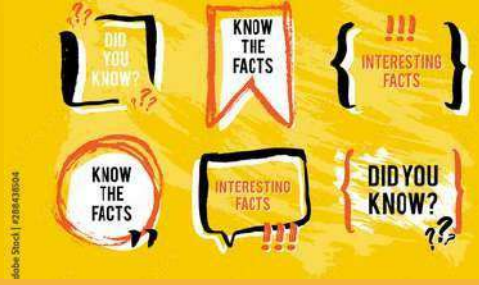


James Batcheller Sumner (November 19, 1887 – August 12, 1955) was an American chemist. He discovered that enzymes can be crystallized, for which he shared the Nobel Prize in Chemistry in 1946 with John Howard Northrop and Wendell Meredith Stanley. He was also the first to prove that enzymes are proteins.It was in 1917 at Cornell where Sumner began his research into isolating enzymes in pure form; a feat which had never been achieved before. The enzyme he worked with was urease, which he isolated from jack beans. Sumner's work was unsuccessful for many years

In 1926 he demonstrated that urease could be isolated and crystallized. He accomplished this by mixing purified urease with acetone and then chilling it; the chilled solution produced crystallized urease. He was also able to show by chemical tests that his pure urease was a protein. This was the first experimental proof that an enzyme is a protein, a controversial question at the time.

In 1937 he succeeded in isolating and crystallizing a second enzyme, catalase. By this time, John Howard Northrop of the Rockefeller Institute had obtained other crystalline enzymes by similar methods, starting with pepsin in 1929. It had become clear that Sumner had devised a general crystallization method for enzymes, and also that all enzymes are proteins.

FUNFACT



Who came up with 10,000 STEPS A DAY Concept

The eminent Greek physician Hippocrates said, “Walking is man’s best medicine”. Fitness freak society seems to have taken this 10,000-step benchmark to heart.

With fitness trackers wearables, smartwatches and smartphone apps, people track their steps throughout the day. Some may even pace around aimlessly to meet this seemingly arbitrary goal.

So… is it really a health obfiective to walk 10,000 steps a day? What’s so special? Why 10,000 Steps? Why not 8000 or even 15000?

Is this magic number determined through RCT? Or Randomly Selected?

Answer is neither. It is a MARKETING CAMPAIGN

This magic number was invented in 1965 as a marketing gimmick when a Japanese company, Yamasa Clock, developed a personal-fitness pedometer called the “Manpo-kei” which translates to “10 000 steps meter”; {“man” meaning 10,000, “po” meaning steps, and “kei” meaning system}.

The Japanese character for 10,000 looks almost like a person walking or running, which is likely how the gadget-maker landed on the name—and the number.

They used it as a clever marketing tool, built a campaign for their new step-tracker off the momentum of the 1964 Tokyo Olympics and it became their slogan. It was trendy, catchy and easy to remember, especially when accompanied by a sketch of a person who’s literally walking.

As it happened, the product was a success, and people were soon striving to achieve their 10,000-step goal each day. Wellness professionals, sports companies and even the health associations supported this trend.

Kindly note: this information is not to discourage walking/running. Walk/flog/run as much as you can. - it surely has health benefits (https://pubmed.ncbi.nlm.nih.gov/35247352/)

Laboratory Touchup

Laboratory Diagnosis of Poisons

Laboratory diagnostics play an important role in the treatment of patients with acute poisoning. The classical clinical chemistry and hematology tests help initiate supportive treatment, and specialized methods enable elucidation of the poisons involved. In this context, from laboratory perspective two facets should be catered:

1. **Estimation of analyte in question** i.e., quantification of the analyte includes:

a. Analytical method adopted

b. Time of sample collection after the exposure to toxin

c. Type of vacuettee/container used for sample collection to rule out any interfering substances

d. Turn Around Time (TAT): It is the time interval from the time of receiving of sample in the laboratory to the release of report.

2. Interpretation of the obtained data

Depending on the severity of the clinical symptoms and the substances involved, intoxications may be very serious events. Management of such patients with potentially serious poisoning requires two different approaches:

1. **Intensive supportive care**, not specific for any particular xenobiotic, rather it caters overall functioning of the human body i.e., physiological state of an organism.
2. **Special care adapted to the poison**.

Considering the two-way approaches for clinical management, laboratory diagnostics is needed in two different fields of activity:

First*, *supportive investigations i.e., laboratory tests need to describe the pathophysiological state of the patient. At a minimum, these include coagulation tests, blood cell counts, blood gas tests, serum electrolytes, liver and kidney function tests, glucose, creatine kinase and osmolality. Except for osmolality, these are very common and fully automated tests which can usually be performed within a very short period of time. With these test results supportive treatment can be initiated.

Second, Specific tests, which in turn are divided into two groups:

Group I: The first group of tests are frequently needed in admitted patients with acute poisoning. At a minimum the list includes, Carboxyhaemoglobin, Cholinesterase (plasma and erythrocyte), Ethanol, Iron, Lithium, Methemoglobin, Paracetamol, Paraquat (qualitative urine test), Salicylate, Theophylline, Valproate, Digoxin.

Group 2: The second group are assays that are important for patient management but which are infrequently needed viz, Carbamazepine, Cyanide, Ethylene glycol, Lead,Mercury, Arsenic, Methanol, Methotrexate, Paraquat (quantitative plasma assay), Phenobarbital, Phenytoin, Thallium, Toxicology screen

Indications for specific assays

The indications for laboratory assays can be summarized as follows

1.To confirm the diagnosis of poisoning when this is in doubt.
2.To influence patient management, e.g. application of a specific therapy; hemodialysis; cessation of treatment.
3.Transfer of the patient to the intensive care unit or
4.Forensic reasons.

Sample collection

For most investigations relevant to poisoned patients, serum or plasma is suitable for analysis. Exceptions are

- ethanol (plasma with fluoride/oxalate [or fluoride/EDTA] anticoagulant),
- carboxyhemoglobin and methemoglobin (heparinized whole blood), and
- red cell cholinesterase, lead and mercury (whole blood anticoagulated with EDTA

Time of Samp le collection

Varies with the analyte in examination (General rule: ASAP with few exceptions).

Analytical Methods:

The most common tests are immunoassays, which have the advantage of being fast and highly automated. These assays are available for the substances which are often involved in intoxications. The other analytical technique which is widely used is hyphenated chromatography consisting of either **high-performance liquid chromatography** or **gas chromatography** as **chromatographic systems and detection with a diode-array or mass spectrometer**.

Few of the most common and well-accepted parameters or procedures used in acute poisoning are described here and summarized in Table 1:

Organo-Phosphorus Poisoning

Source: Insecticides which are widely used in agriculture to control pests, weeds, or plants diseases.

- The OP compounds likely to have more adverse effects in developing countries like India due to its easy availability and less awareness leading to high morbidity and mortality.
- Intact organophosphates cannot be detected in the blood due to rapid hydrolysis by the liver. Therefore, the most commonly used test to confirm acute organophosphate poisoning is measurement of cholinesterase activity.

Cholinesterase

- Plasma cholinesterase activity is simpler to measure and is inhibited more rapidly in poisoning. However, reduced activity is less specific and may also occur in genetic pseudocholinesterase deficiency (suxamethonium apnoea), early pregnancy, liver disease, malignancy and hypo-albuminemia.
- Measurement of the red cell cholinesterase activity is more specific and may be used to confirm severe organophosphate or carbamate poisoning when the diagnosis is in doubt.
- Red cell cholinesterase activity is usually reduced to <50% with clinically significant poisoning and to <10% in severe cases.
- Plasma cholinesterase falls and recovers more rapidly after exposure than red blood cell (RBC) cholinesterase. It may take 90–120 days for RBC cholinesterase to recover to normal values.

Ethylene glycol(1,2 ethanediol) and methanol

- Urgent measurement of plasma or serum ethylene glycol or methanol concentration is essential for optimum management.
- It is reasonable to restrict use of these assays to; a) patients who give a history of substantial ingestion; b) patients with suspected severe toxicity as evidenced by metabolic acidosis, especially pH 60 nmol/L) in the presence of an increased anion gap, with or without an increased osmolar gap, when a reliable history is unavailable; In these cases, antidotal treatment should be commenced pending the result of the assay.

- A raised osmolar gap may be present in the early stages of poisoning. Due to the molecular weight of ethylene glycol, and the normal variability of osmolar gaps between patients, toxin concentrations of ethylene glycol may be present without the osmolar gap being greater than normal. An increased osmolar gap is consistent with poisoning but a normal gap does not exclude it.
- It may take several hours for toxic organic acids to be formed from ethylene glycol and methanol. Therefore, the anion gap may be normal in the early stages of methanol and ethylene glycol poisoning, even when life-threatening amounts have been ingested.
- Ethylene glycol is metabolized to oxalic acid which forms complexes with calcium, so close monitoring of the plasma calcium concentration is required. ·Similarly, creatinine and electrolytes should be checked daily as severe renal impairment may occur with ethylene glycol poisoning.

Cyanide Poisoning

- Results from laboratory analysis are very unlikely to be available rapidly enough to influence the acute management of an individual patient.
- However, analysis of samples may inform management of further cases in the event of multiple casualties and also for forensic purposes.
- Estimated/Identified by: LCMS/MS

g-Hydroxybutyrate (GHB)

- GHB is often used as a drug of abuse or as a date-rape drug.
- It has a very short elimination half-life, which allows detection in serum or plasma for about 6 h, and in urine for about 12 h only.
- GHB usually is determined with a specific GCMS/HPLC method after derivatization.

Carboxyhaemoglobin

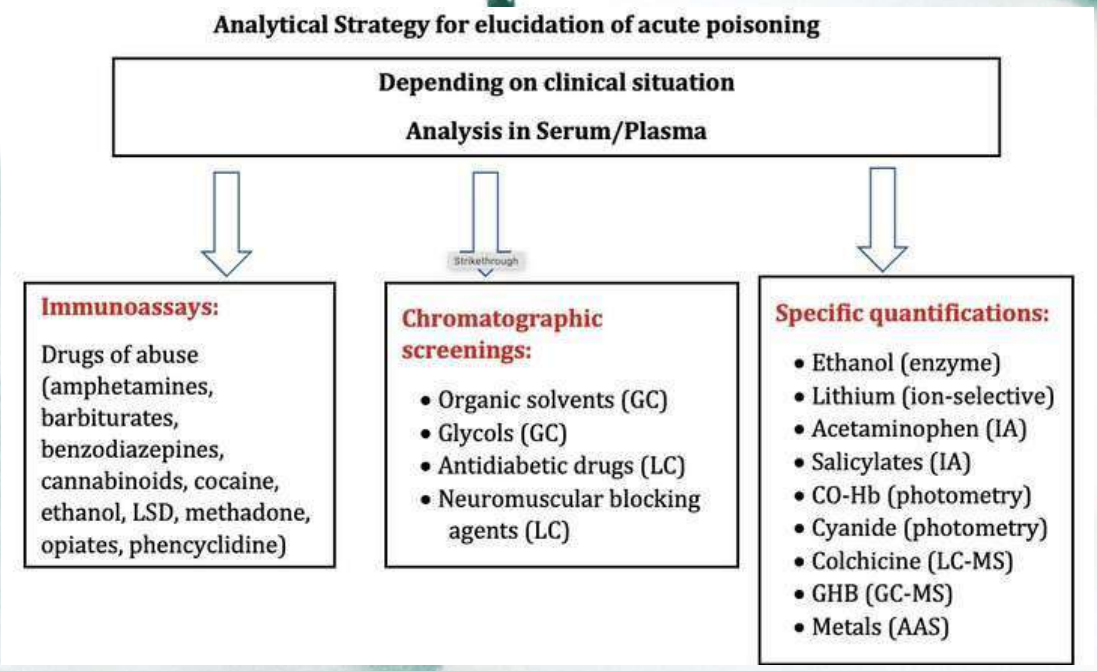
- Carboxyhaemoglobin should be measured urgently in all patients with suspected carbon monoxide poisoning (including those with suspected smoke inhalation).
- It may also be elevated in patients who have ingested methylene chloride.
- A carboxyhemoglobin percentage of >30% total haemoglobin indicates severe poisoning. However, concentrations less than this do not exclude significant exposure, and the relationship between carboxyhemoglobin and severity of poisoning and/or clinical outcome is poor especially when sampling occurs late after exposure.
- Management should be determined by the clinical condition of the patient rather than the carboxyhemoglobin concentration.

Organic solvents

- Besides the popular use of ethanol, the use of organic solvents in intoxication has several reasons: a) may be drunk mistakenly if it was stored in a beverage bottle, b) self-produced liquors erroneously contain methanol, c) may be used as poisons in suicide attempts, d) abuse of solvents by sniffing.
- ·During the first phase after intake of solvents, these compounds add to the osmolality, and their presence and the amount taken can be estimated by calculating the osmolal gap.
- **The organic solvents can be determined by GC coupled to different detectors (e.g., flame ionization or mass spectrometry.**

Table 1. Summary of use of assays of toxins.

Parameter	Indication	Sample Collection time after exposure	Repeat samples	Clinically significant concentrations
Carboxyhaemoglobin	Suspected CO or smoke inhalation	Immediate	No	>20%
Digoxin	Severe digoxin toxicity	Immediate	No	>2.0 mg/L (2.6 nmol/L)
Ethanol	• Undiagnosed coma, with widened osmolar gap. • Severe intoxication; being considered for dialysis.	Immediate	2 hourly samples	1000–1500 mg/L for use as an antidote. 1800 mg/L for significant toxicity (in the absence of other agents)
Iron	• >20 mg/kg elemental iron ingestion within 6 h. • Symptoms suggesting iron toxicity at any time after overdose.	>4h after overdose	After 2hour of initial collection	3 mg/L (55 mmol/L)
Lithium	Suspected acute or chronic lithium poisoning	Immediate or After 6 hr for asymptomatic acute overdose.	6–12 hourly in severe poisoning	Target range 0.4–1.0 mmol/L (12 h post dose
Methaemoglobin	Exposure to relevant toxins	Immediate	Worsening symptoms	>20%
Paracetamol	Suspected paracetamol overdose.	>4 hr	Rarely required	
Paraquat (urine spot test)	Suspected paraquat exposure	Immediate	At 4 hour	Positive indicates the need for plasma analysis
Salicylate	• Salicylate overdose (suspected >120 mg/kg). • Ingestion of methylsalicylate or salicylamide. • Suspected case	>2 h if symptomatic. >4 h if asymptomatic.	2 hour	Serious toxicity usually associated with concentrations >350 mg/L (>2.5 mmol/L)
Theophylline	Clinical features suggesting theophylline toxicity	Immediate if clinical features present. >4 h if asymptomatic	Repeat 2–4 hourly in patients with severe poisoning or concentration >60 mg/L	Serious toxicity usually associated with concentration >60 mg/L (>333 mmol/L)



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Quote of the day

Happier thoughts lead to essentially a happier biochemistry. A happier, healthier body.

John Hagelin

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