The importance of biochemical pharmacology in the interpretation of forensic toxicological analysis

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Professor Francis Camps wound up the symposium on Forensic Toxicology held at the Chemical Defence Establishment, more than 10 years ago in 1972. Speaking on the future of forensic toxicology he said "This is easy: at the moment it has none". He made the proviso that Forensic Toxicology will have a future only if the basic knowledge of Physiology, Anatomy and Pharmacology is used to interpret the results of examination of autopsy specimens for drugs and poisons.

This paper presents some examples of how the interpretation of values of drug levels found in various compartments of the body requires an understanding of Pharmacology. In order to do this it is proposed to look at the following areas:—

a) the distribution of toxic substances in the body
b) individual variations
c) interactions
d) the possible significance of biochemical test

Distribution of Drugs

Toxic absorption of Digoxin resulting from a change in formulation sparked an interest in the phenomenon of bioavailability. Numerous studies have been carried out to find out how much of a particular drug is absorbed from various dosage forms. This involved the development of analytical procedures for the determination of drugs in low concentrations. Study of how the drug distributed in various body compartments in turn evolved into the science of Biopharmaceutics and Pharmacokinetics. This knowledge of the distribution of drugs can be important in the interpretation of forensic results.

For example a 22 year old tourist was taken to hospital in a coma but she was certified dead on arrival. Routine examination of the blood taken at autopsy revealed no toxic substances. However analysis of a liver sample yielded high concentrations of dextropropoxyphen. A bottle of 100 Distalgesic tablets dispensed to the patient on the previous day was later found empty among her personal effects. It was also later learnt that she had had a history of unsuccessful suicide attempts.

LD50 in Humans!

From blood level — time curves in animals one can determine the toxic level as well as the lethal level. The lethal level may be of more interest to the forensic scientist. These levels are influenced by the subject's individuality such as weight, age, sex, genetics and metabolism; drug interactions and tolerance could also change these levels. In the case of Theophylline the therapeutic level is 5ug/ml whereas the toxic level is 12ug/ml. In such a case can a forensic scientist decide that a patient has died from Theophylline overdosage if the blood level is say 20ug/ml? Questions often asked of a forensic scientist include:—

1) Was a drug or poison taken?
2) If yes, had the drug been used in that person for therapeutic purposes?
3) What was the dosage involved?
4) Can an estimate of the time of administration be given?

In clinical practice one might determine when a drug was administered from the biological half life of the drug. However in post mortem cases one often does not have sufficient details to give an interpretation of the results from a blood level determination. The problem that one has to consider is that the value for the lethal level cannot be fixed at a specific level for a particular drug. In other words one cannot fix the lethal level in a clear cut table for all drugs. In basic pharmacolgy an attempt was made to establish LD50. This in itself is limited as it only shows at what dose 50% of the animals are killed. One hopes of course that the day will not come when one could determine the LD50 for Humans! So the Forensic scientist has an added problem in comparison with the clinician in that controlled experiments on humans in the relevant dosage ranges cannot be carried out. It is difficult to relate in court the determined levels of a particular drug with the effect that those levels are likely to have had on the individual concerned.

Individual Variations

In the classical case of alcohol it is easy to say that a sample of blood had so many milligrams percent of alcohol. But it is much more difficult, if not impossible to answer such questions as "How much alcohol was drunk by the victim?", "When was it drunk?", "Was the victim in a state of intoxication?" As is well known this depends on a large number of factors including how tolerant the individual was, at what rate was it ingested, what were the stomach contents at the time of ingestion and so on; data which are usually not available to the Forensic Scientist. Has the victim had a massive haemorrhage? Has the individual been admitted to hospital and been given a large dose of I.V. fluilds? If the subject has been given medical treatment then this may well influence the interpretation of results. In fact there may be much to be said for the statement that the individual's ability to walk a straight line may be more relevant than GC results, if such a test can in fact be carried out on the individual.

Again, what is the lethal dose for Propranolol? Is the published figure of 10ug/DL in blood applicable to a patient who was previously suffering from bradycardia? Would it not be reasonable to suppose that in such a patient a much smaller dose would still be lethal? The same may be said for Digoxin in Hypokalaemia, for Tolbutamide in Hypoglycaemia and for Codeine, Morphine and Meperidine in the case of respiratory impairment.

It is worth considering the case of Trimethoprim and Theophylline. One could predict the dose from blood level standards on computer, provided that certain data are available, e.g. time when drug was taken. This hold well for Trimethoprim as there is little subject to subject variation. In the case of Theophylline per rectum the actual dose is not easily determined. This is due to the enormous subject to subject variation in rectal absorption. In fact in recent times it has become common practice that in order to keep a patient between the minimum effective concentration and the toxic level, the clinician "titrates" the necessary dose and schedule of administration for the particular patient by monitoring the blood level of the drug.

The factors which should alert the Forensic Scientist to possible problems in absorption include:—

a) a sparingly soluble drug used in large doses
b) long acting dosage forms
c) gastric contents or flora affect the drug
d) drug is absorbed by active transport
e) different formulations and dosage forms

Drug Metabolites

Another important aspect in Forensic Toxicological investigations is the question of drug metabolites. Beckett states that "the biological activity of a drug is frequently the result of a complex series of reactions involving both metabolites and the parent drug". Consequently in order to determine the toxic effects of a drug which could lead to lethal consequences, it is essential to have an understanding of:—

1) the metabolic routes involved in the change of drugs in the body
2) the physico-chemical and biological properties of the metabolites

The lack of stability of the metabolites in biological fluids especially in post mortem samples leads to great difficulties especially when quantitative analysis is required. In addition analytical artifacts could further complicate matters. On the other hand, a knowledge of the rate constants involved in the metabolic process together with a quantitative determination of metabolites could be of great value when interpreting results. Was the Victim under the influence of drugs during an accident? An evaluation of metabolites could assist in answering such a question. The amount of normeperidine in meperidine overdosage could help to show when the drug was taken.

In a recent murder case in Malta, the accused pleaded possible mental influence from lead poisoning. The accused was a lead worker. The Court ordered the examination of the accused in order to examine this plea. It is to be borne in mind that a toxic lead level at the time of examination does not necessarily imply that the same was the case at the time of commission of the crime. This is a case where the toxicology of lead presents difficulties. In such a case one has to determine lead values as well as consider the biochemical effects of lead, for example, the effect of lead on the biosynthetic pathway of haem. Delta amino laevulinic acid synthetase (d-ALA synthetase), delta aminolaevulinic acid dehydrogenase (d-ALA dehydrogenase) and haem synthetase are inhibited by lead. This leads to increased urinary excretion of D-ALA, coproporphyrin and sometime porphobilinogen and the accumulation of protoporphyrins in erythrocytes. Only the presence of these abnormalities points to slow lead poisoning over a period of time.

Biochemical Tests

It is interesting to see how the forensic scientist could use other biochemical data as signs of toxicity. A number of drugs can alter the normal values of biochemical substances and detecting the presence of such abnormalities will alert the investigator to look into the possibility of the presence of the drugs that cause them.

Examples of drugs altering Blood Urea Nitrogen and Serum transaminases and Phosphatases are given in the tables 1 and 2.

Table 1 — DRUGS EFFECTING SERUM TRANSAMINASES AND PHOSPHATASES

Cabamazepine	Nicotinic acid
Methyldopa	Oral contraceptives
Tricyclic antidepressants	Papaverine
	Sulphonamides

Table 2 — DRUGS AFFECTING BLOOD UREA NITROGEN

a) drugs changing protein metabolism
   i) anabolic: Thyroid, insulin Testosterone
   ii) catabolic: corticosteroids
b) drugs acting directly on kidney aminoglycosides and tetracyclines methoxyfluorane (autopsy revealed calcium oxalate crystals in renal tubles) diuretics

If postmortem studies are to be carried out to examine the effects of drugs on biochemical tests such as blood urea nitrogen (BUN) and serum transaminases and phosphatases, the effect of death itself on the normal values given by these biochemical tests must first be studied. A blood sample was taken from a rabbit and the BUN and VMA were determined. The rabbit was sacrificed and the blood samples taken at fifteen minute intervals from the time of death were analysed. No significant changes were observed up to three hours after death.

A number of drugs are known to increase VMA levels following administration. This may be useful both from the point of view of colloborating other evidence as well as a possible indicator of drugs to be looked for. It is difficult for example to establish death due to excessive isoprenaline in halation from the toxicological point of view. A patient found dead with two empty medihaler-iso by his bedside was shown to have a high value of VMA. This corroborated the evidence that a high dose of isoprenaline had been inhaled since increase in the VMA level is usually small when small doses are administered but may increase significantly with large doses. If a high VMA level is found it may be worth the effort to look for Insulin. Although the increase is not so large as to indicate Phaeochromocytoma, it is statistically significant.

The level of a xenobiotic in an organ and its relation to toxicity may differ from person to person, especially in cases of tolerance or genetic variation. In determining the cause of death, it would be of interest to the forensic scientist if a method could be found to establish whether the person had developed tolerance to the drug found in his body. Using a number of mice, it can be shown that mice which developed tolerance to morphine had higher concentrations of calcium in the brain than those which were given morphine for the first time.

The Interpretation of Laboratory Results

It is true that a routine analysis can never be replaced in Toxicolgy. The literature gives many examples of cases where there was no clue to the possible toxic substance present if any, and where the routine analysis lead to significant discoveries. However in some cases, the victims symptom's and history much be ascertained because a careful analysis of these may point towards looking for a specific poison or class of poisons. The absolute level of a drug found in a body fluid can be of very limited value if considered in isolation. What is of significance is the likely effect that such a level of drug would have had on the behaviour or physiological reaction of the particular individual. In a number of cases it is not easy to state unequivocally whether a level of a drug in a biological fluid is lethal, toxic or in the therapeutic range. This is the grey area where the interpretation of laboratory results is what really matters. This is the area which requires a good knowledge of physiology and pharmacology.

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