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Radioimmunoassay: its Application to Drugs of Abuse

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Abstract. The possibilities offered by the technique of radioimmunoassay in detection of drug abuse are briefly reviewed. The laboratory procedure for utilizing antibodies is reviewed and some examples given of various systems utilizing antisera in the analytical quantitation of various drugs of abuse in biological material.

Key Words
Drug abuse detection
Radioimmunoassay

Pharmacology is a hybrid science resulting from cross correlation of biochemical and physiological data. The dynamics and kinetics of drug actions must, therefore, be measured by tools and techniques of the parent sciences. Likewise, the definition of drug action must be interpreted in the terms of either physiological or biochemical principles. The development of pharmacology as a science has progressed in accordance with the strides made in the technology of the analytical tools used as sensors in the pharmacological experiment.

In the area of neuropharmacology some progress has been made in developing analytical tools for defining the mechanism of action of drugs which act on the central nervous system, but despite this concerted effort the progress noted has fallen short of defining in biological terms the specific mechanism of action of drugs at either the functional or behavioral level. This gap in knowledge is nowhere greater than in the area of drugs of abuse. After years of studying heroin addicts, for example, the scientist is still unable to define physical dependence in biological terms. The problem of psychological dependence is, likewise, no closer to solution. It appears that the scientist awaits the development of more sensitive and precise tools before further progress can be made in this area. One developing technique which seems to have application to pharmacodynam-

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ics, especially in the biological research areas related to drugs of abuse is the radioimmunoassay (RIA).

Radioimmunoassay, first used in the measurement of hormone concentrations in body fluids by *Berson and Yallow* (1959), involves simple laboratory procedures and instrumentation. The concepts and the terminology, however, may require a little study since they are outside the background of the classically trained pharmacologist or chemist.

An excellent review article by *Skelley et al.* (1973) treats the terminology, history and kinetics of RIA techniques as well as precision, sensitivity and specificity. Their article is oriented toward the use of the procedure for measuring hormone concentrations in body fluids.

Laboratory Procedure

The application of immunoassay procedures to drugs in body fluids is a relatively recent development. Essentially, three major steps are involved in an RIA procedure.

First, antibodies (Ab) must be generated in a suitable animal. Usually rabbits, goats, or sheep are used for this purpose. Since most drugs are small molecules and are not immunogenic *per se* the drug is complexed chemically to a carrier substance, a protein, to make it antigenic (*Landsteiner*, 1946). Bovine or human serum albumin has been successful for this purpose. The drug in this complex is called the hapten and the entire complex, the antigen (Ag). The Ag, suspended in Freund adjuvant, is injected at weekly intervals and the animal generates antibodies to both the carrier and the hapten. These can be separated from each other in the antisera by standard methods.

The Ab in the antisera are reacted with small amounts of the drug complex by standard methods resulting in the antigen-antibody complex, forming a small precipitate which is not readily visible. Therefore, some method must be used to mark the drug so that it can be found in the system. This constitutes the second problem. One approach is to attach a short chain of amino acids to the drug. Tyrosine moieties in the polymer may be tagged with ^{125}I. Also, it may be possible to 'tag' the drug directly. A standard curve can then be constructed by reacting graded amounts of the tagged drug with antisera. The resulting plot, counts per minute versus log concentration of drug, shows a linear relationship, as would be expected.

The third step involves addition of biological fluid containing unlabelled drug to the Ab-labelled drug system. The unlabelled and labelled drugs compete for the active binding sites on the Ab – essentially an isotope dilution procedure. More detail about the immunologic and assay technology may be found in a review article by *Spector* (1973).

Systems Using (Ag-Ab) Reaction

To date the RIA technique has been worked out for many drugs but, more specifically for three major drugs of abuse – morphine, pentobarbital and lysergic acid diethylamide (LSD).

Spector and Parker (1970) reported the production of antibodies to morphine. The antibodies were able to detect nanogram quantities of morphine. A publication by *Spector* (1971) gives details of the analytical procedure. The standard used in this method is (^{3}H) dihydromorphine which binds to antibodies produced in rabbits. Unknown amounts of unlabelled drug in the urine or plasma of drug users will compete with the labelled compound for the active binding sites on the antibody. A linear relationship can be seen if one plots percentage inhibition of binding of labelled drug versus amount of non-labelled drug. Unknown quantities can be read off the standard curve directly. The sensor here is, of course, the radioactivity in counts per minute. The method is sensitive to 100 picograms (pg) morphine. The Ab to morphine also recognizes normorphine, codeine and heroin but not the 3-mono-glucuronide of morphine. Therefore, the method has high sensitivity and good, but not absolute specificity. However, the time required for the assay precludes its application to the testing of a large number of samples.

Immunological techniques for measuring morphine in body fluids have employed sensors other than radioisotopes. *Leute et al.* (1972) have 'tagged' the hapten in their test system with a free radical instead of a radioisotope. Morphine labelled with nitroxide is allowed to react with rabbit antimorphine antisera. A capillary tube containing these components is placed in the cavity of an electron spin resonance spectrometer. As in the RIA system, the unlabelled morphine in the serum and/or urine of a user competes with the free radical labelled morphine for the binding sites on the Ab. As might be expected, the specificity in this system is the same as with the RIA since the antibodies are generated with the same antigen. The sensitivity of the method is such that a 50-percent inhibition is achieved at 2.85 μg/ml. This method requires very small amounts of test material and is very rapid. A special instrument (FRAT–Syva Company, 3221 Porter Avenue, Palo Alto, Calif., USA) is required, however, and there is some evidence of high percentages of false positive data, if careful laboratory procedures are not followed.

The same Ab to morphine has been used in another system by *Adler and Lui* (1971) and *Adler et al.* (1972). The sensor for this method is inhibition of agglutination of red blood cells. Specificity of this procedure is the same as for *Spector*'s RIA and the FRAT methods; the sensitivity is in the 10^{-2} nanogram range. However, here no expensive equipment is necessary. Testing is rapid and involves only standard laboratory techniques. Results appear to be reliable and reproducible with a very low incidence of false positive data. *Catlin et al.* (1973)

have modified the original *Spector and Parker* method (1970) and demonstrated the applicability of the modified method to the rapid analysis of urine samples from known chronic heroin addicts. This modification utilizes goat antisera and the report details the method for preparing large numbers of reaction tubes for storage up to 6 months.

Antidrug sera have been employed in another system involving an enzyme and its substrate as the sensor. The technique worked out by *Rubenstein et al.* (1972) involves the attachment of the drug to an enzyme at, or close to, its active substrate binding site. The enzyme drug complex is inactive. Antidrug sera is added together with the specific substrate for the enzyme. The antibodies pull the drug away from the enzyme leaving it free to act on the substrate. Urine is added to the test system. It if contains drug this drug will compete with the drug attached to the enzyme for the active sites on the antibody. Not all the enzyme will be free and action on the substrate will be limited. Usual standard curves may be constructed from which drug concentrations in unknown urine samples can be determined. Lysozyme is the enzyme used in the system and the substrate is a solution of bacteria – *Mycococcus lysodeikticus.* The enzyme causes lysis of the bacteria when it is released from drug.

This system has been developed for morphine, methadone, cocaine, barbiturates, and amphetamine (*Rubenstein et al.*, 1972). It eliminates the isotopic marker and hence the need for expensive equipment. The method has been suggested as a rapid, easy and practical procedure for screening a large number of urine samples in drug rehabilitation facilities and treatment centers. It appears to have some assets and potential, but its serious deficit is its lack of specificity. For example, when present in concentrations of from 1–10 μg/ml the following barbiturates will give readings equivalent to 2 μg/ml of secobarbital, mephobarbital, pentobarbital, amobarbital, phenobarbital, thiopental, Tolbutal, butabarbital. Morphine detection by this system is clouded by the fact that concentration of 0.35 μg/ml codeine, 5.5 μg/ml of heroin or nalorphine give readings equivalent to 0.5 μg/ml of morphine. Likewise with the amphetamines, there are about six compounds in concentrations of less than 10 μg/ml which give readings equivalent to 30 μg/ml amphetamine. As a research tool it would probably need some modification since its sensitivity at present is in the μg/ml range.

Other methods are available for analysis of the barbiturates. Classical analytical methods for identifying specific barbiturates in biological material are either time consuming or lacking in sensitivity. With respect to the drug abuse problem it would be advantageous to be able to differentiate by a simple technique whether the 'barbiturates' in the urine of users consisted of secobarbital, amobarbital, pentobarbital, or a mixture of all these, or others. Gas chromatographic procedures have this capability but are not practical for a large number of samples. *Spector and Flynn* (1971) have developed an RIA utilizing rabbit antisera to pentobarbital. The antibodies bind equally well to barbital and phenobarbital.

Therefore, substitution on the C-5 position of the barbiturate molecule is not discriminated by the antibody. On the other hand hexobarbital and thiopental are bound by the antibody to a much lesser degree. This method is rapid and should be useful, according to the authors, for determining barbiturate concentration in biological material.

Two major drugs of abuse, LSD and THC, have until recently, defied the analytical chemist. Since only microgram quantities of LSD are ingested by the abusers for psychedelic effects it has been virtually impossible to monitor either blood or urine levels of LSD and/or its metabolites for metabolic distribution or disappearance data. Recently *Taunton-Rigby et al.* (1973) have succeeded in developing an RIA for LSD. The method is sensitive to 0.01 pmol LSD in biological material and is extremely specific. The antisera have been tested against some 35 drugs with molecular structures similar to LSD and cross reactivity does not occur at concentrations adequately sensitive to detect LSD. The antibodies have been generated in rabbits and the tagged antigen and the antisera needed for the assay are commercially available (Collaborative Research, Inc., 1365 Main Street, Waltham, Mass., USA).

Taunton-Rigby and Kelley are currently working on the development of a similar method for THC. Antibodies have been developed in rabbits and, at present, tests are in progress to define specificity of the antisera.

Conclusion

These immunologic analytic procedures have far reaching application. They have a role to play in research areas where metabolic studies and plasma concentrations of drug need to be correlated with behavioral parameters. Programs involving the screening of urine for drugs will profit by the use of such methods. This is especially true in methadone maintenance clinics, where drug use other than methadone, needs to be identified. Because of their specificity, these methods are potentially effective tools in the hands of the forensic chemist and will also be useful in the emergency rooms of hospitals. Here the problem of identifying the presence of drugs is a serious one in a patient involved in an accident where corrective surgery may be indicated. Care must be exercised lest the prescribed analgesic and/or anesthetic synergize with a previously self-administered drug.

In the research area there is wide application for techniques which have the ability to measure drug concentrations in biological material with quantitative precision and high specificity. The interest of many investigators in utilizing RIA gives testimony to its potential for supplying much-needed factual information, especially in the area of drug abuse problems.

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