WHAT IS THE FUTURE OF LIQUID SCINTILLATION COUNTING (LSC) IN CLINICAL CHEMISTRY AND IN CLINICAL RESEARCH?

Edwin D. Bransome, Jr.
Lawrence B. Hendry

Departments of Medicine & Endocrinology
Medical College of Georgia
Augusta, Georgia

Virendra B. Mahesh

Department of Endocrinology
Medical College of Georgia
Augusta, Georgia

C. Robert Baisden

Department of Pathology
Medical College of Georgia
Augusta, Georgia

The use of liquid scintillation counting in clinical laboratories and for biomedical research has increased greatly over the more than 25 years that commercial LS counters have been available. Vibrating reed electrometers and gas-filled ionization detectors for the measurement of tritium and carbon 14 respectively have been supplanted by LSC. Other commonly employed isotopes for which LSC is now the preferred method are: ^{32}P, ^{35}S, ^{45}Ca, ^{55}Fe, and the α-emitting actinides (McDowell and Weiss, 1976). Other isotopes counted by LSC have recently been listed by Gibson (1976), Bransome and O'Conner (1978), and Soini (1978).

Copyright © 1980 by Academic Press, Inc.
All rights of reproduction in any form reserved.
ISBN 0-12-549902-7

We are in agreement with Painter (1976) who described LSC as an "unreliable and unpredictable technique in the clinical laboratory" because of practices leading to spurious results. We in fact address one of the problem areas in this volume (O'Conner and Bransome, 1980). Partially because of such problems, most clinical radioimmunoassays are carried out using antigens labelled by ^{125}I or ^{131}I, isotopes usually measured more economically by solid (NaI) crystal scintillation counting. The impact of recent innovations in instrumentation for measuring radionuclides, such as Cerenkov and semiconductor detectors (eg. GeLi, Si) is at present uncertain (Table I).

TABLE I. Techniques of Measuring Isotopic Labels

A. Equipment for Radionuclides	Status
Ionization (gas-filled) detectors	Superceded
Inorganic scintillation detectors (NaI, CsI)	Competitive
Other organic scintillation detectors (plastic, crystals)	Noncompetitive
Cerenkov detectors	Potential LSC can be used
Semiconductor detectors (e.g. GeLi, Si)	Potential
B. Equipment for Stable Isotopes	
Gas chromatography-mass spectrometry (GC-MS)	Competitive
High performance liquid chromatography mass spectrometry (HPLC-MS)	Potential

The future of LSC in medical and biomedical laboratories is further compromised by competitive nonradioisotopic techniques, some of them quite new and possibly unfamiliar to the reader. One of the purposes of this paper is to consider applications of these methodologies to current provinces of radioisotopes methodology.

STABLE ISOTOPES COMPETITIVE WITH LSC

Several approaches to in vivo studies of pharmacokinetics, hormone metabolism or intermediary metabolism can be carried out, not with radioisotopes, but with stable isotopes (eg.

deuterium, ^{13}C) the abundance of which (in comparison to specific unlabelled atoms) can be measured by mass spectrometry. Table I refers to mass spectrometer-data system combinations available commercially which have been interfaced to a technique for separation or purification, either gas-chromatrography (GC) or high performance liquid chromatrography (HPLC). Where as radioisotopes may be most appropriate for dilution analysis, it is frequently necessary in metabolic studies to obtain a very accurate gravimetric measurement of the labelled substance of interest. This is usually unnecessary with mass spectrometry inasmuch as it is possible to measure atomic (isotope:nonisotope) ratios very accurately.

Stable radioisotopes measured by mass spectrometry offer another advantage for studies of the metabolism of drugs or hormones. As illustrated for the synthetic estrogen mestranol in Figure 1, administration of a 1:1 mixture of the deuterated and unlabelled ligand of interest will yield metabolites with "twin peaks" the normal ions and an equal population of ions two units larger (mestranol-D_2). Metabolites heretofore unidentified can be identified through a programmed scan of the mass spectra of chromatographic fractions, by the presence of "twin peaks".

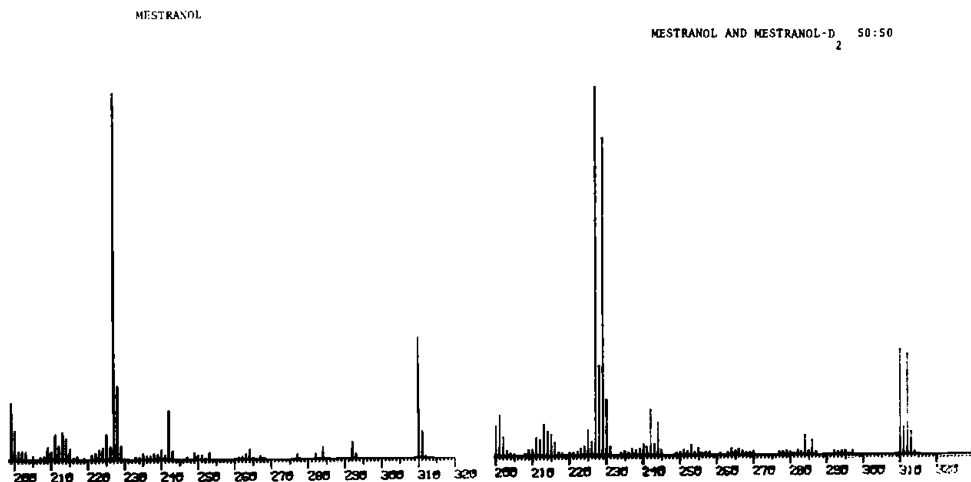

FIGURE 1. Electron impact mass spectra of mestranol from 220-320 AMU. The upper panel shows ions from the unlabelled estrogen, the lower panel from a 1:1 mixture of deuterated and unlabelled mestranol. Direct probe analyses with a Finnigan 1015-D quadrupole GC-MS system. (See the text for discussion.)

IMMUNOASSAYS

Radioimmunoassays (RIA's) which were introduced into clinical laboratories twenty years ago (Yalow, 1978) represent a type of specific and sensitive saturation analysis. Table II outlines the two types of RIA and two parallel approaches to enzyme immunoassay, a technique introduced in the early 1970s (Miedema et al., 1971; Rubenstein et al., 1972; Bastiani et al., 1973; Voller et al., 1976a).

TABLE II. Type of Immunoassay

A. *Radioimmunoassay*

Competitive Inhibition - Reaction of ligand and antibody. Measurement of ligand**

Immunoradiometric - Reaction of ligand and 1st antibody. Measurement of 2nd antibody to 1st antibody.*

B. *Enzyme Immunoassay*

EMIT^R homogenous enzyme assay - Reaction of ligand-enzyme conjugate with antibody to ligand. Free ligand increases enzyme activity (NAD $\rightarrow$ NADH).

ELISA - Enzyme linked immunosorbent assay - Reaction of antibody - enzyme conjugate with ligand. Free ligand or antibody affects enzyme activity (NAD $\rightarrow$ NADH).

Reagents for the analysis of a number of drugs and hormones have recently become available commercially from the Syva Company (Palo Alto, Ca) as EMIT^R homogenous enzyme immunoassays. Table III lists the clinical tests currently available from Syva which do not require prior separative steps.

TABLE III. Some Clinical Assays Which Can Be Performed By the EMIT^R Technique

Urine test for drugs of abuse			
Opiates	*Barbiturate*	*Benzodiazepine*	*Cocaine*
Amphetamine	*Methadone*	*Propoxyphene*	
Blood levels of drugs and hormones			
Digoxin	*Primidone*		*Ethosuximide*
Lidocaine	*Thyroxine*		*Carbamazepine*
Phentoin	*Procainamide*		*Theophylline*
Phenobarbital	*N-Acetyl procainamide*		*Methotrexate*

The ligand of interest is complexed to an enzyme (eg thyroxine to malate dehydrogenase). Procedures for the assays involve: addition of a solution to denature serum proteins (where appropriate), brief incubation, addition of specific antibody to the ligand along with the coenzyme nicotinamide adenine dinucleotide (NAD) and mixing followed by addition of the enzyme substrate and the ligand-enzyme complex. The complex inhibits enzyme activity; reaction with antibody increases enzyme activity and therefore the conversion of the coenzyme NAD to NADH which can be monitored with a spectrophotometer set at 340 NM. Ligand in the serum or urine samples competes with the enzyme-ligand complex for antibody. The more ligand, the less interaction of antibody and complex, and the less enzyme activity. Enzyme activity reflected by optical density, at 340 NM is thus inversely proportional to the concentration of ligand.

The virtues of this appraoch are that the equipment is relatively inexpensive and widely available, that the procedure can be automated, that results can be obtained on the same day, that the reagents are stable for months, and that the per assay cost of reagents is low (less than $2). Problems include the technical difficulty of synthesizing active enzyme conjugates, less sensitivity than RIA (in part because of steric hindrance of the enzymes), and the statistical problems which affect precision when any saturation assay is performed under non-equilibrium conditions.

ELISA or enzyme-linked immunosorbent assays originated from the use of enzyme-antibody (eg. horse-radish peroxidase) complexes for immunohistochemistry. The same principles of spectrophotometric measurement have been employed for the measurement of antibodies (eg. Engvall, 1976; Leinikki and Passila, 1976) or the detection of viruses (Voller et al., 1976b).

ALTERNATIVE TO IMMUNOASSAY

Three of the reasons for using radioimmunoassays are: sensitivity (the ability to measure very small amounts of a molecule), specificity of the measurement, and the ability to measure without having to purify the molecule of interest. Recent technical developments indicate that many radioimmunoassays will in time be supplanted by direct chemical measurements. A pervasive problem with RIA, the frequent lack of identity of biological and immunological activity, may be avoided, as fractions quantitated can be collected and tested for biological activity.

Luminescence Assay. Measurement of a number of ligands of biomedical interest (see Table IV), sometimes with great sensitivity, is discussed in this volume and has been discussed at LSC conferences for the last decade. Single photon counters or LS counters may be employed. This technology has not been adopted in many medical laboratories; because of the lack of specificity, careful separation and purification of the ligands of interest is therefore necessary. The advent of new HPLC procedures (see below) could result in greater popularity of this approach.

TABLE IV. Luminescence Assays

Type of Molecule	*Examples*	
Ions	*Ca, Co, Cr, Cu*	
Inorganic small molecules	*NH , HCl, H O , N O*	
Carcinogens	*nitrosamines*	
Metabolites	*glucose*	*3-hydroxybutyrate*
	pyruvate	*ATP*
	glycerol	*cyclic nucleotides*
	malate	*hematin*
	uric acid	
Drugs		
Vitamins	*B-12, biotin*	
Coenzymes	*NAD, NADH, NADPH, FMNH*	
Enzymes	*LDH, reductases, oxygenases*	
Cellular activity	*platelet adhesion, phagocytosis*	

Gas Chromatography. The widely used technique of GLC for separation of volatile organic or volatile derivatives of non-volatile compounds is capable of considerable sensitivity, but lacks specificity. Mass spectrometry (see Ligon, 1979) has added specificity to this chromatographic technique as well as sensitivities in the attomole (10^{-18}) range. (See Figure 2) (Hunt and Crow, 1978).

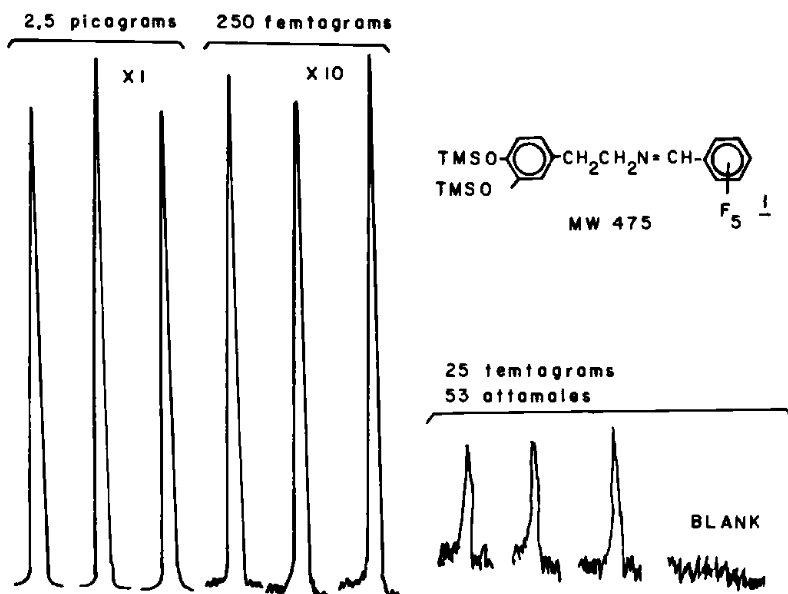

FIGURE 2. Response obtained by monitoring the molecular ion (ml/e = 475) of dopamine derivative under GC-MS conditions with the instrument operating in the single ion monitoring (SIM) mode. Signals correspond to three successive injections of 2.5 pg, 250 fg, and 25 fg samples, respectively.

High performance liquid chromatography. The use of HPLC over the last decade, primarily for the analysis of drugs, is now rapidly evolving because of the development of new columns and the introduction of new methods of detection: fluorimeters and electrochemical detectors, (Snyder and Kirkland, 1979). Figure 3 provides an example of protein separation on a new column support introduced this year by Waters Associates (Milford, Mass.) HPLC-Mass Spectrometry is in its infancy compared to GC-MS but has even greater potential for the clinical or biomedical laboratory (Arpino and Guiochon, 1979). Figure 4 illustrates the use of HPLC-MS to differentiate a drug from one of its metabolites.

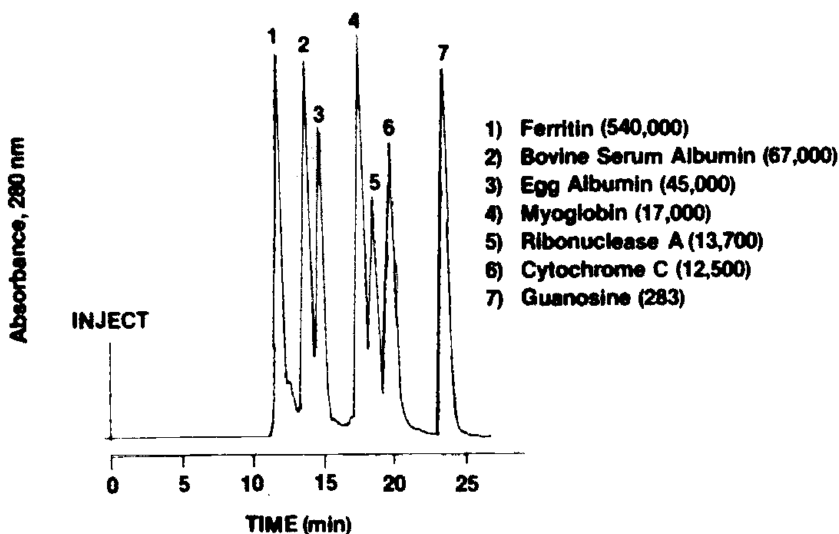

FIGURE 3. This shows a typical separation of a complex protein mixture achieved by using dual protein columns (Waters Associates) and decreasing solvent flow rate.

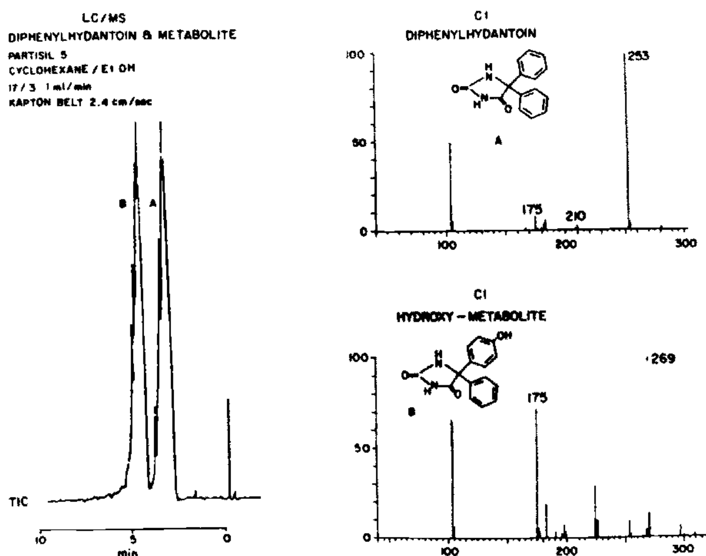

FIGURE 4. LC/MS analysis of diphenylhydantoin and metabolites.

CONCLUSIONS

We suggest that over the next decade the majority of new analytical methods introduced into clinical and biomedical laboratories will involve chemical technology that does not involve liquid scintillation counting. The cost of equipment (Table V), especially of mass spectrometers may be a limiting factor for a while.

TABLE V. Comparison of Equipment

	Detection	Cost ($)
Luminescence Photometer	?	∿ 7000
Spectrophotometer for EMIT Assays	$\sim 10^{-6}g$	∿ 9000
Gas-Liquid Chromatograph (GC)	$\sim 10^{-6}g$**	10-15,000*
High Performance Liquid Chromatograph (HPLC)	$< 10^{-6}g$**	10-30,000*
Scintillation Counter	NA	15-25,000*
GC-Mass Spectrometer (GC-MS)	10^{-9}-$10^{-15}g$**	70-180,000*
HPLC-Mass Spectrometer (HPLC-MS)	10^{-9}-$10^{-12}g$	∿ 140,000*

* *Cost will vary with accessories and type of data system.*
** *Sensitivity will vary with the detector system and the specific application.*

There will of course continue to be a place for radioisotope methodology, especially for dilution and activation analysis, and some of the radionuclides will continue to be measured to greatest advantage by liquid scintillation counting.

REFERENCES

Arpino, P.J. and Guiochon, G. (1979). *Anal. Chem. 51*, 682A.
Bastiani, R.J., Phillips, R.C., Schneider, R.S., and Ullman, E.F. (1973). *Am. J. Med. Tech. 39*, 211.
Bransome, E.D. and O'Conner, J.L. (1978). "Liquid Scintillation Counting", (M.A. Crook and P. Johnson, eds.) p. 3, Heyden, London.
Engvall, E. (1976). *Lancet ii*, 1410.
Gibson, J.A.B. (1976). "Liquid Scintillation Science and Technology" (A.A. Noujaim, C. Ediss, L.I. Wiebe, eds.), p. 153, Academic Press, New York.

Hunt, D.F. and Crow, F.W. (1978). *Analyt. Chem. 50 (13)*, 1781.
Leinikki, P. and Passila, S. (1976). *J. Clin. Path. 29*, 1116.
Ligon, W.V. (1979). *Science 205*, 151.
McDowell, W.J. and Weiss, J.F. (1976). "Liquid Scintillation Science and Technology", (A.A. Noujaim, C. Ediss, L.I. Wiebe, eds.) p. 17, Academic Press, New York.
Miedema, K. Boelhouwer, J. and Otten, J.W. (1971). *Clin. Chim. Acta 40*, 187.
O'Conner J.L. and Bransome, E.D. (1980). This Volume
Painter, K. (1976). In "Liquid Scintillation Science and Technology" (A.A. Noujaim, C. Ediss and L.I. Wiebe, eds.) p. 69, Academic Press, New York.
Reubenstein, K.E., Schneider, R.S. and Omen, E.F. (1972). *Biochem. Biophys. Res. Commun. 47*, 846.
Soini, E. (1978). *Science Tools 25*, 38.
Snyder, L.R. and Kirkland, J.J. (1979). "*An Introduction to Modern Liquid Chromatography*", Wiley-Interscience, Englewood Cliffs.
Voller, R. Bidwell, D.E. and Bartlett, A. (1976a). *Bull. World Hlth. Org. 53*, 55.
Voller, A., Bartlett, A. and Bidwell, D.E. (1976b). *J. Gen. Virol. 33*, 165.
Yalow, R.S. (1978). *Science 200*, 1236.