Fluorescent Excitation Analysis for In Vitro Tests

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Use of fluorescent excitation analysis for in vitro tests eliminates patient exposure to radiation. Tests already developed measure extracellular fluid volume, red cell volume, and glomerular filtration rates. These compare favorably with alternate techniques.

The applications and usefulness of fluorescent excitation analysis (FEA) in performing some in vitro tests currently using radioactive labels or chemical analysis of stable elements are outlined. FEA is fast, accurate, and cost effective and avoids exposing the patient or attending personnel to radiation. This can be extremely important when studying children, pregnant women, normal volunteers, and when performing serial studies on the same subject.

Technique

When an in vitro test is performed using a radioactive tracer, the patient receives a certain amount of radiation associated with that tracer. Attending personnel are also exposed. Since all counting is performed *outside* the patient, e.g., in a well counter, it would be ideal if the tracer could be rendered "radioactive" just during counting. FEA is a technique that achieves this in a convenient way.

When an element is bombarded by a beam of gamma ray or x-ray photons with an energy higher than the binding energy (BE) of the K-shell electrons in an atom, ejection of these electrons occurs, thereby generating a vacancy in the K-shell. This vacancy is subsequently filled by either an L-shell or M-shell electron. In this process the atom emits photons of two different and unique energies, Kα and Kβ, which are called the characteristic radiations and are unique to each element (Fig. 1). The same process can occur in other electron shells, producing lower energy characteristic x-rays with lower yield. The process of exciting stable elements to emit characteristic radiations is termed fluorescent excitation (1).

The exciting photons can also undergo Compton scattering, producing secondary photons of an energy lower than that of the exciting (original) beam, with the

FLUORESCENT EXCITATION
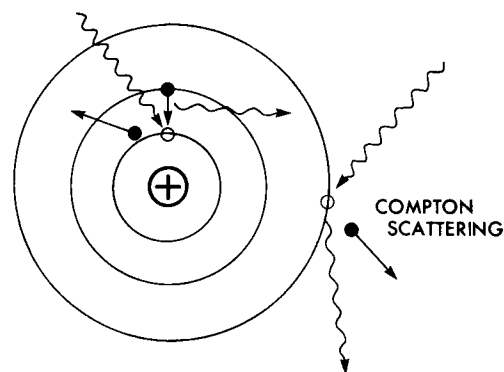
FIG. 1. Process of fluorescent excitation: photon of sufficient energy ionizes one of inner-shell electrons. Vacancy thus generated is filled by higher shell electron, which emits x-rays of fixed energy in process. Compton scatter, partial transfer of energy to outer electron, can also occur.

exact value of that energy depending on the scattering angle. Detectors with very precise energy resolution are needed to separate the fluorescent radiations from each other and from all other radiations reaching the detector (mainly Compton scattering). Thus, lithium-compensated silicon detectors [Si(Li)] are commonly used since they achieve energy resolutions in the order of 400 eV FWHM for the x-rays of iodine. This excellent energy resolution is due to the direct and efficient manner in which the energy of incoming x-rays is transferred into charge (a 30-keV x-ray produces a signal consisting of 10,000 electrons that are fully collected). For NaI, on the other hand, the incoming photon energy is transferred first to one electron, which then produces secondary ionization that generates light. Only a fraction of this light reaches the photomultiplier tube, at which point charge is once again produced (Fig. 2). For a 30-keV x-ray, only 10–30 electrons are produced at the cathode of the tube. Energy resolution
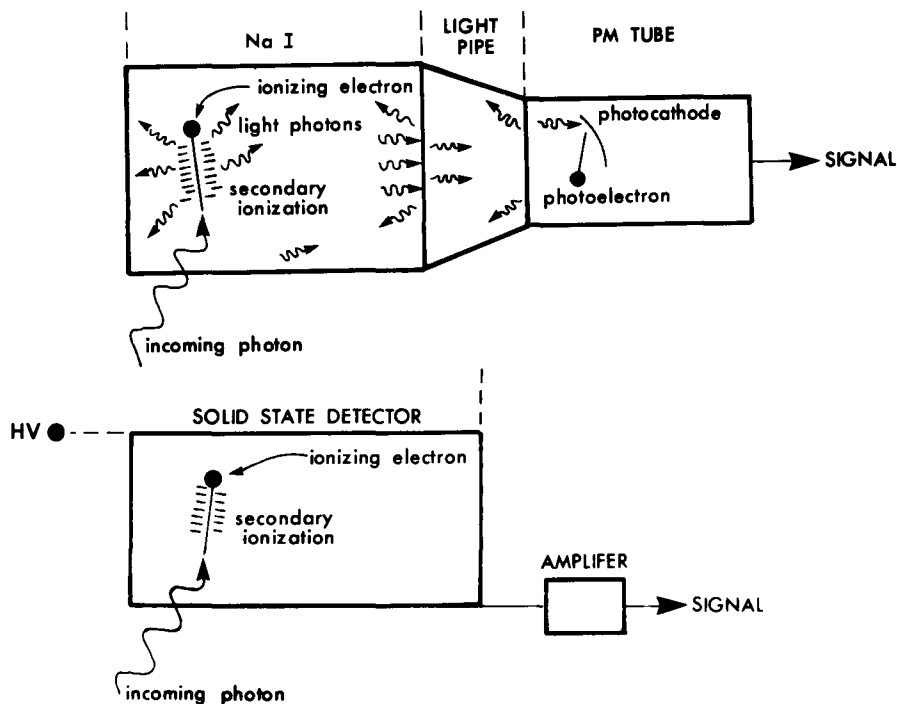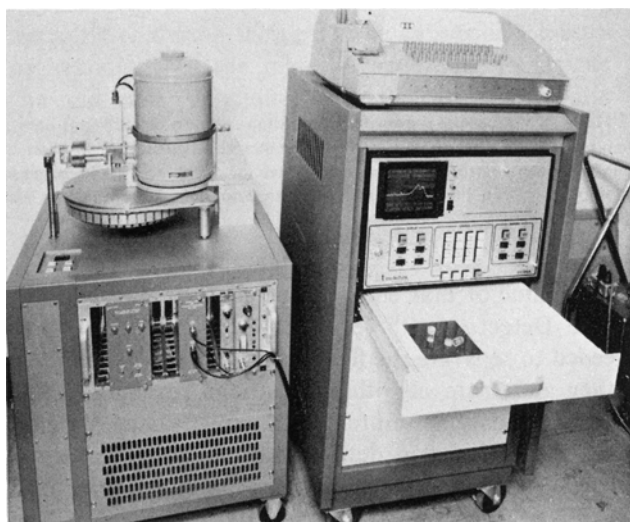
FIG. 2. Process of signal generation in NaI is complex and very inefficient: Only 10–30 quanta of information are produced by 30-keV primary photon. In contrast, charge collection in Si(Li) detector is direct, and 10,000 quanta of information are produced in detection of 30-keV photon.
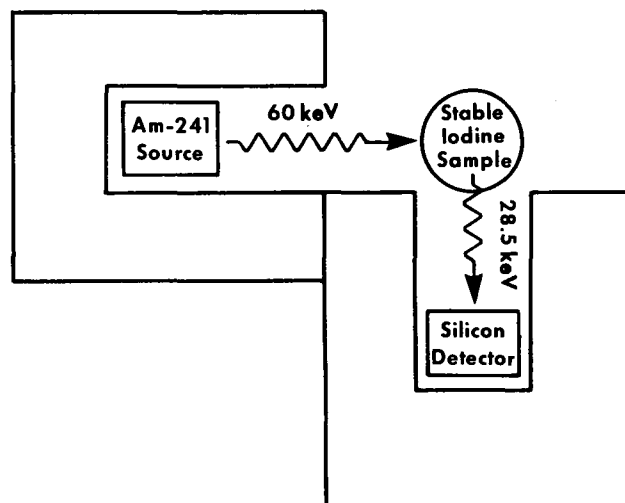

FIG. 3. Automated FEA system, with sample changer on right, which includes Si(Li) detector on top and analyzer on left.

FIG. 4. Excitation arrangement shown schematically. Sample is placed in sensitive volume defined by intersection of collimators.

in Si(Li) far surpasses that of NaI and makes the former critically valuable in practical FEA systems.

Equipment

Figure 3 illustrates the present automated system, which has been developed in collaboration with the Lawrence Livermore Laboratory (Radiochemistry Division, Livermore, Calif.), the KeVex Corp. (Analytical Products Division, Burlingame, Calif.), and Ino-Tech, Inc. (Madison, Wis.). It consists of an optimized Si(Li) detector, an automated special sample changer, and a programmable pulse height analyzer. An encapsulated [241]Am (60 keV) source is used for iodine and cesium FEA, and [109]Cd (22 keV) is used for bromine FEA. The sample changer holds 48 2-cc vials and has a mixing mode prior to analysis.

Figure 4 shows schematically the geometry in which the sample is analyzed. Source and detector are collimated at 90 deg to each other, and the sample is placed in the sensitive volume defined by the intersection of the collimators. A typical spectrum for an iodine-containing sample is shown in Fig. 5. Tracer concentration in the sample is calculated using the formula:
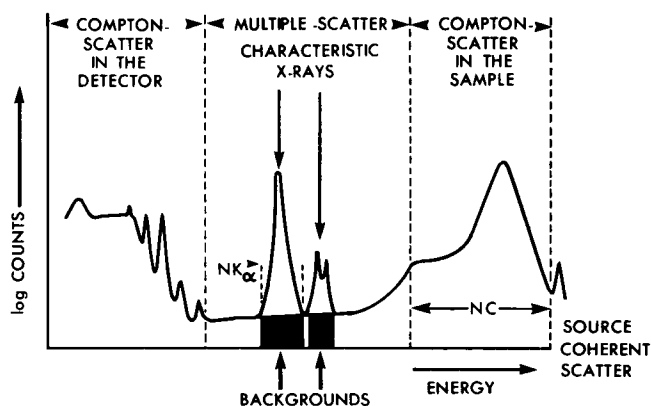
FIG. 5. Typical spectrum from iodine-containing sample excited by ^{241}Am.

$$\text{Conc} = K[(NK/NC) - B]$$

where NK and NC are the counts in the $K\alpha$ and Compton channels, respectively, B is NK/NC for a pure water sample, and K is obtained by measuring NK and NC for a sample of known concentration. Concentration determinations are independent of the volume of the sample and have been shown to be linear (K and B constant) from 1 ppm to 2-4% in concentration. The results are also independent of dead time and exact sample positioning and can be expressed in any desired units of concentration (e.g., mEq/liter, mg/gM, ppm).

The wide range of linearity is most important in medical applications in which low-concentration biologic samples have to be compared to high-concentration injectates. FEA has been applied to several nuclear medicine tests, replacing radioactive labels with stable ones. Three of the tests that we will discuss in this context are extracellular fluid volume (ECFV), red cell volume (RCV), and glomerular filtration rate (GFR).

Results

The bromide ECFV has been measured in the past either with ^{82}Br or by chemical assay (Conway's microdiffusion technique) of stable bromine (2). Radioactive labels present obvious disadvantages both in isotope decay (i.e., limited shelf-life) and in exposure to the subject. Chemical analysis of bromine is performed by a trained technologist and is limited to determinations of only two patient studies per day. Using stable bromine and FEA all four samples needed for a single patient determination (injected standard, preinjection plasma, and 3- and 4-hr plasma samples) can be analyzed in 5 min (Table 1).

RCV determinations were done in a series of patients using both ^{51}Cr and stable cesium chloride (CsCl$_2$) to label autologous red cells (3). The labeling procedure was facilitated by the addition of nystatin. The procedure followed the conventional technique of radioactive labeling of red cells with the exception of extra saline washes after the incubation period. The routine

10- and 40-min samples were counted using FEA and the RCV was calculated using the following formula: RCV = [Injectate weight × (Cs conc in injectate) × (fractional hematocrit)] ÷ [(specific gravity of blood) × (Cs conc in blood sample)]. Table 2 shows the comparison of the radioactive (^{51}Cr) versus the nonradioactive techniques (CsCl$_2$). As can be seen, there was excellent agreement between the two.

A reliable and simple determination of GFR would aid in the clinical evaluation of renal disease and its progress during therapy. Inulin clearance thus far has been the most reliable method for measurement of GFR. However, this technique requires 4-8 hr of technologist time to set up and to analyze samples for one patient.

The use of radioactive ^{125}I-iothalamate is also accepted for GFR determinations. Again, this technique is expensive, and repeated determinations are limited due to radiation exposure to the patient. Figures 6 and 7 show GFR results obtained using Conray-60 (stable meglumine iothalamate) and FEA that compare with clinically accepted clearances (4). The Conray-60 technique is performed much the same as the
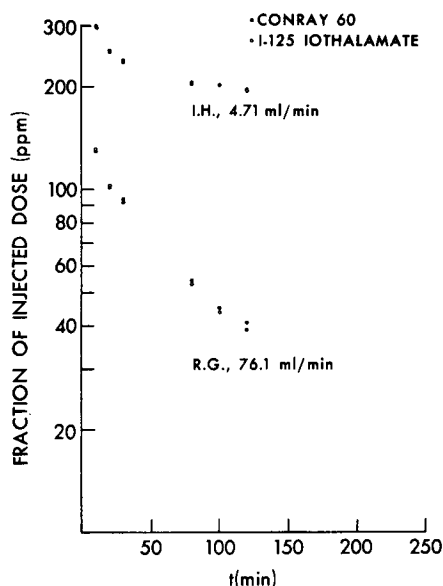
TABLE 1. System Accuracy for Bromine (April 1975)

Count time	Concentration range (mEq/liter)	Reproducibility (%)
30 sec	500 to 75	$\sigma \lesssim 1$
1 min	~9	$\sigma \simeq 1.2$
	~1.3	$\sigma \simeq 2$
2 min	~0.5	$\sigma \simeq 4$
15 min	~0.075	$\sigma \simeq 5$

Clinical range: *Injectate* $\simeq$ 300 mEq/liter; plasma levels $\simeq$ 1-2 mEq/liter.

Chemical analysis: $\sigma \simeq$ 2.6%, eight samples/8-hr day.

TABLE 2. Human Red Cell Volumes

		Time after injection				
	10 min			40 min		
	^{51}Cr	Cs		^{51}Cr	Cs	
Tracer	Volume (liter)	Volume (liter)	Difference (%)	Volume (liter)	Volume (liter)	Difference (%)
1	2.30	2.44	6.1	2.24	2.15	− 4.0
2	1.69	1.64	−3.0	1.71	1.73	1.2
3	0.75	0.71	−5.3	0.76	0.78	2.6
4	1.39	1.45	4.3	1.41	1.46	3.5
5	1.10	1.16	5.5	1.08	1.33	23.1
6	1.35	1.38	2.2	1.35	1.35	0.0
7	1.01	0.98	−3.0	1.01	1.00	− 1.0
8	3.46	3.26	−5.8	3.56	3.41	− 4.2
9	2.29	2.22	−3.1	2.22	2.24	0.9
10	1.24	1.29	4.0	1.28	1.36	6.3
11	2.91	2.93	0.7	2.99	2.96	− 1.0
Mean			0.2			2.5
s.d.			4.4			7.5
p			<.001			<.001

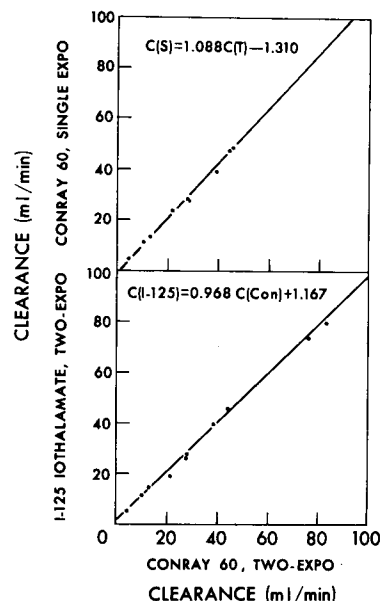FIG. 6. Clearance of ^{125}I-iothalamate and Conray-60 from patients with high and low GFR. Overlap was exact where only one data point is shown.

FIG. 7. (Top) Comparison of Conray-60 GFR values using two- and single-exponential analysis. (Bottom) ^{125}I-iothalamate and Conray-60 GFR values in patients calculated by two-exponential analysis.

[125]I-iothalamate, with the advantages of low cost per study and no radiation exposure to the patient. As can be seen, only four blood samples are necessary, a preinjection, and 2-, 3-, and 4-hr after injection. This replaces the more involved techniques that require eight blood samples and two-exponent analysis.

Conclusion

The results thus far have demonstrated that FEA of nonradioactive tracer elements can be applied routinely to many in vitro tests. The advantages of this technique over radioactive or chemical analysis have been discussed and include increased accuracy, low cost, and, most importantly, the absence of radiation exposure to the patient. This is of particular importance in making these studies available to broad population groups.

Acknowledgments

References

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