Photon Activation Analysis of Toxic Elements

I have read with interest Lisk's account of several analytical techniques available today for the study and monitoring of toxic elements in biochemical and geochemical systems (*1*). While discussing the sensitivity of the technique of neutron activation analysis (NAA) for trace element analysis in various samples, Lisk points out that NAA "cannot be used to determine certain important elements such as lead." My purpose in this technical comment is to point out that photon activation analysis (PAA), a technique not mentioned by Lisk, can be used for the detection of Pb and other elements in different types of sample matrices.

For several reasons, PAA has not received as much attention in the past as NAA. But recent technological developments in the area of nuclear detectors and analyzers have paved the way for the use of PAA in various sample matrices. Hislop and Williams (*2*) have used PAA to analyze samples of the lunar materials returned on the Apollo 12 mission and other biological materials. They used the bremsstrahlung radiation from the Harwell 45-Mev linear accelerator (linac) for activation of the samples and a high-resolution, solid-state nuclear spectroscopy system for the analyses of the complex γ-ray spectra. They reported that PAA is particularly valuable for the determination of Fe, Ca, Ti, Sr, Zr, Cs, Sb, Tl, As, Rb, Pb, Hg, and Ni. By using chemical separation of the irradiated samples, they were able to obtain sensitivities as high as 0.02 μg. Kato and Oka (*3*) used the 300-Mev linac at Tohoku University for the PAA of 52 elements (chemical purity, 99.9 percent or better). Sensitivities for PAA with 30- and 60-Mev bremsstrahlung were reported by them for 13 elements which have nuclear properties unfavorable for NAA. Lutz (*4*) has presented a review of PAA with emphasis on its use in biological, geochemical, oceanographic, and forensic matrices. He has listed calculated sensitivities for 60 elements after activation with photons having energies of 25, 30, and 35 Mev. Aras *et al.* (*5*) have reported the use of PAA for the quantification of 14 elements present in urban particulate materials. The elements assayed include, among others, Pb, As, Cr, Ni, and I. They also have discussed the advantages, reproducibility, accuracy, and sensitivity of PAA for both light and heavy elements in various matrices.

Kuttemperoor (*6*) has studied the photon activation of several elements including Pb, using the bremsstrahlung radiation from the 25-Mev betatron at the Milwaukee School of Engineering. The photonuclear reactions $^{204}Pb(\gamma,n)^{203}Pb$ or $^{204}Pb(\gamma,\gamma)^{204m}Pb$ can be used for the detection and unambiguous identification of trace amounts of Pb in various samples. The reaction $^{204}Pb(\gamma,\gamma)^{204m}Pb$ was used by Chattopadhyay and Jervis (*7*) for the determination of Pb in soil samples. They irradiated the samples with 35-Mev x-ray photons and determined the activity of ^{204m}Pb having an energy of 0.375 Mev and a half-life of 67 minutes. Concentrations of the order of 10 parts per million (ppm) have been reported with the use of a Ge(Li) detector without chemical separation, and detection limits of about 0.001 μg may be achieved by radiochemical separation of ^{203}Pb prior to γ-ray detection (*7*). Lutz (*8*) has used PAA to determine the concentrations of Pb in biological and environmental samples. He irradiated the samples using the electron accelerator at the National Bureau of Standards, inducing the photonuclear reaction $^{204}Pb(\gamma,n)^{203}Pb$. The most prominent γ-ray from ^{203}Pb (energy, 0.279 Mev; half-life, 52.1 hours) was used for the identification of Pb. Sensitivities of the order of 0.3 ppm in beef liver and 43 to 47 ppm in orchard leaves were reported by Lutz. Bryan *et al.* (*9*) and Guinn (*10*) have studied the activation of forensic samples by PAA. They have analyzed several samples of moonshine whiskey and have reported Pb concentrations of the order of 4 to 80 mg per liter of whiskey.

The technique of PAA is nondestructive and is favored over NAA and atomic absorption for the detection of Pb. It is also preferred in the detection of a number of other biologically important elements such as C, O, Si, N, F, Mg, Fe, and Ni. Another advantage of PAA over NAA for biological samples is that one can avoid the creation of ^{24}Na due to the presence of ^{23}Na which is very abundant in any biological sample.

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References

1. D. J. Lisk, *Science* **184**, 1137 (1974).
2. J. S. Hislop and D. R. Williams, *Publ. AERE-R 6910* (Her Majesty's Stationery Office, London, 1971).
3. T. Kato and Y. Oka, *Talanta* **19**, 515 (1972).
4. G. J. Lutz, *Anal. Chem.* **43**, 93 (1971).
5. N. K. Aras, W. H. Zoller, G. E. Gordon, G. J. Lutz, *ibid.* **45**, 1481 (1973).
6. V. Z. Kuttemperoor, *Mater. Eval.* **32**, 153 (July 1974); ——— and R. A. Kobiske, *Int. J. Appl. Radiat. Isotopes*, in press; V. Z. Kuttemperoor, in preparation.
7. A. Chattopadhyay and R. E. Jervis, *Radiochem. Radioanal. Lett.* **11**, 331 (1972).
8. G. J. Lutz, *Radioanal. Chem.* **19**, 239 (1974).
9. D. E. Bryan, V. P. Guinn, D. M. Settle, *AEC Rep. GA-7041* (Atomic Energy Commission, San Diego, Calif., 20 March 1966).
10. V. P. Guinn, *AEC Rep. GA-8013* (Atomic Energy Commission, San Diego, Calif., 20 October 1967).

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Infant Hue Discrimination?

Fagan (*1*) concluded that infants 4 to 6 months of age are capable of hue discrimination. He based his argument on their preference for fixating checkerboard patterns composed of Munsell squares chosen to differ in hue but equated for brightness and saturation, as opposed to unpatterned targets of either hue. In addition, he reported a Kendall rank correlation coefficient of .82 between the percentages of total fixation to patterned targets and his index of hue differences. However, there are problems associated with both the stimulus array and the data analysis that seriously weaken his conclusion.

In order to create a checkerboard pattern varying in hue but not in brightness, Fagan used pairs of Munsell squares of different Hue but of the same Value. In the Munsell system, stimuli of a given Value have the same luminous reflectance, Y in terms of the Commission Internationale de l'Eclairage (C.I.E.) designation. Thus, other things being equal, samples of the same Value appear approximately equal in lightness. This is true for infants, however, only if their luminosity function is a close approximation to the C.I.E. photopic luminosity function, which was determined for adults. The study cited by Fagan to support this possibility utilized such a restricted wavelength range (450 to 650 nm, in 50-nm steps) that it cannot be regarded as conclusive (*2*). In addition, the yellow, crystalline lens of the eye probably absorbs differently in infants than in adults (*3*). On this basis alone it is improbable that adults and

Table 1. Measured tristimulus values and chromaticity coordinates of the Munsell samples used in Fagan's study (*1*).

Nominal notation	Light source	Tristimulus values			Chromaticity coordinates		Renotation Hue
		X	*Y*	*Z*	*x*	*y*	
5R 5/6	C	24.92	19.99	16.44	.406	.326	5.6R
	A	32.66	22.99	4.88			
5G 5/6	C	13.26	19.00	17.10	.269	.385	5.3G
	A	13.67	17.18	5.50			
5B 5/6	C	16.13	19.67	36.88	.222	.271	5B
	A	14.39	16.93	11.51			
5YR 5/6	C	22.51	19.48	9.30	.439	.380	5YR
	A	29.99	22.27	2.92			
5PB 5/6	C	19.65	19.80	40.85	.245	.247	4.9PB
	A	18.58	18.22	12.03			
10R 5/6	C	23.99	19.63	12.36	.429	.351	0.2YR
	A	31.53	22.74	3.71			
10G 5/6	C	13.80	19.68	21.45	.251	.358	0.1BG
	A	13.69	17.45	6.89			
10B 5/6	C	18.55	20.42	40.51	.233	.257	0.1PB
	A	17.06	18.19	12.14			
2.5G 4/10	C	5.80	11.75	6.75	.239	.484	2.7G
	A	5.98	9.94	2.50			
7.5G 4/10	C	5.72	11.90	10.93	.200	.417	7.7G
	A	5.22	9.56	3.89			

infants have precisely the same spectral sensitivity (*4*).

Even assuming that the infant luminosity function is identical to the C.I.E. photopic luminosity function, Fagan's results are difficult to interpret because of the illuminant that he used. Samples of the same Value appear approximately equal in lightness only if the illuminant is a good approximation to C.I.E. illuminant C (artificial daylight) since the Munsell system is calibrated with such illumination. Fagan used "high-intensity incandescent bulbs," which are much closer in spectral irradiance to C.I.E. illuminant A than to C. Since A is considerably weaker in energy in the short-wave region of the spectrum than is C, luminous reflectance (*Y*) of colored chips equated for C will be different for A. This effect can be clearly seen in Table 1 (for chips taken from the same production lot as those Fagan used) by comparing the *Y* values under the two illuminants. In general, *Y* is larger for reddish chips and smaller for bluish chips under illuminant A.

Another problem associated with Fagan's stimulus conditions is the chromatic adaptation induced by the light blue surround. It must be assumed that the infants are adapted to it. Thus, the stimuli are specified with respect to a "blue-rich" illuminant (C), but they are illuminated with a lamp that is relatively "blue-poor" (A); and the "blue-sensitive" mechanism of the eye is reduced in sensitivity because of the blue adapting field. All of these factors combine at the short-wave end of the spectrum: stimuli that reflect short waves are rendered dark compared to those that reflect middle or long waves. The net effect is that the shifts in *Y* shown in Table 1 must be regarded as conservative estimates of brightness changes.

Thus, Fagan's infants could have been discriminating partly (or entirely) on the basis of brightness cues. In deciding the question, it is necessary to have at least a rank index of both brightness and hue differences. Brightness differences can be expressed on an ordinal scale by differences in the *Y* values. Fagan used differences in the tristimulus value *X* as a measure of hue differences. This is not an adequate measure, as discussed below, but for comparison with the original analysis I shall temporarily adopt it.

Table 2. Ranks associated with each sample-pair pattern for fixation preference (F.P.); *X* differences under illuminant C, $X_C = |X_1 - X_2|_C$; *X* differences under illuminant A, $X_A = |X_1 - X_2|_A$; *Y* differences under illuminant A, $Y_A = |Y_1 - Y_2|_A$; and renotation Hue differences under illuminant C, $H_C = |H_1 - H_2|_C$.

Pattern	F.P.	X_C	X_A	Y_A	H_C
5YR-10G	1	4	4	3	4
5R-5B	2	3	2	1	1
10R-10G	3	2	3	5	2
5R-5G	4	1	1	2	3
5R-5PB	5	5	5	4	5
5G-5B	6	7	11	11.5	6
5B-5PB	7	6	6	6	7
5R-5YR	8	9	7.5	8	8
5B-10B	9	8	7.5	7	9
5R-10R	10	10	9	11.5	12
5G-10G	11	11	12	10	11
2.5G-7.5G	12	12	10	9	10

Table 2 lists the pairs of samples that Fagan used along with the rank order of the percentages of total fixation to patterned targets. The fourth column is the rank order of *X* differences for illuminant A ($|X_1 - X_2|_A$) determined from the measurements shown in Table 1. The Kendall rank coefficient between the percentages and the *X* differences is .656 (*5*). The rank coefficient between the percentages and the *Y* differences for illuminant A ($|Y_1 - Y_2|_A$), shown in column five, is .595. Both values are significant at $P = .01$. In addition, the two factors, *X* and *Y*, are highly correlated (Kendall rank coefficient of .815). Consequently, it is impossible to choose between these two factors related to hue and brightness as the determining covariable of preferential fixation.

The choice of *X* differences as an index of hue differences is, however, highly questionable. In the C.I.E. system, *X* is calculated by integrating the product of the spectral irradiance of the lamp, the spectral reflectance of the sample, and the color matching function $\bar{x}$. The latter is a complex function with the major peak at 600 nm and a secondary peak at 440 nm (*6*). Thus, there is no simple relation between $\bar{x}$ and hue differences; the same is true for *X*. An example demonstrates this clearly: equal-energy, monochromatic lights of 430, 460, 540, and 650 nm are easily discriminable in terms of hue yet they have approximately the same *X* values when reflected from a white surface.

A better scheme is simply to take the renotation differences in Hue, as the Munsell renotation was designed to reflect equal steps in hue. Renotation Hue was estimated based upon the measured chromaticity coordinates, shown in Table 1, and the charts of the renotation system (*6*). Here again, it is necessary to use illuminant C in order for the equal spacing aspect to hold; the use of illuminant A would certainly distort the Munsell color space to some unspecified degree. Yet, given the choice presented by Fagan's data, it is probably the best index of hue differences available. We can hope that the ranks of the true renotation Hue differences with illuminant A are not too far from those with illuminant C. The last column in Table 2 shows the ranks of the renotation Hue differences ($|H_1 - H_2|_C$)

calculated from the Munsell renotation, which assumes illuminant C. The Kendall rank correlation coefficient between percentage fixation and renotation Hue differences is .819, which is highly significant. Recall that the correlation for percentages and Y differences was .595. In addition, the two factors are highly correlated (Kendall rank correlation of .682). Thus, even with a better index of hue differences than Fagan used, there is little evidence for asserting that hue, and not brightness, is the determining variable of the observed fixation preferences. Until all of these factors are properly controlled, the high correlation between percentage fixation and renotation Hue differences can only be regarded as suggestive evidence that infants are capable of hue discrimination.

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References and Notes

1. J. F. Fagan III, *Science* **183**, 973 (1974).
2. D. Trincker and I. Trincker, *Albrecht von Graefes Arch. Ophthalmol.* **156**, 519 (1955).
3. Y. Le Grand, *Light, Colour and Vision* (Chapman & Hall, London, ed. 2, 1968).
4. There are at least two other potentially serious problems associated with spectral sensitivity that make Fagan's results difficult to interpret. (i) There is a great deal of variation among adults with regard to spectral sensitivity, especially in the short-wave region. If infants are similarly variable, brightness matches made on the basis of any single curve are unlikely to hold for particular individuals. It might be argued that in a large enough sample, such effects would be averaged out. This is incorrect since mismatches in either direction could provide brightness cues on which discriminations could be made. (ii) The C.I.E. photopic luminosity functions, which the Munsell system utilizes, underestimate luminosity in the short-wave end of the spectrum [C. H. Graham, Ed., *Vision and Visual Perception* (Wiley, New York, 1965)]. Hence, for any given Y, samples reflecting short waves should be somewhat lighter than those reflecting predominantly middle and long waves.
5. Fagan reported a Kendall rank correlation coefficient of .82 between the percentages and the X_C differences. The measurements shown in Table 1, however, indicate that his ranks of 8 and 9 (5R-5YR and 5B-10B) should be reversed. The actual correlation would then be .758.
6. G. Wyszecki and W. S. Stiles, *Color Science: Concepts and Methods, Quantitative Data and Formulas* (Wiley, New York, 1967).
7. I thank L. A. Riggs, K. D. White, and J. C. Armington for improving the manuscript. I also thank the Munsell Color Co. and L. Galyon, who made the measurements shown in Table 1.

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Wooten has shown that differences in brightness covaried, to some extent, with hue differences for the checked patterns I presented to infants. In his reanalysis of my data he reports a correlation of .819 between hue differences and percentage fixation. He concludes, however, that "there is little evidence for asserting that hue, and not brightness, is the determining variable of the observed fixation preferences," since hue and brightness differences correlated .682 and brightness differences correlated .595 with preferences. Wooten's conclusion is premature.

Methods of partial correlation provide a statistical answer as to whether the observed relation between hue differences and preferences was due to variations in brightness. Using the correlations reported by Wooten, I obtain a Kendall partial rank correlation coefficient of .703 between hue differences and preferences when the effect of brightness is held constant. In other words, there is a substantial correlation between hue differences and percentage fixation which cannot be attributed to brightness differences. On the other hand, the partial correlation between brightness differences and preferences is virtually zero at .086 when hue differences are held constant. Hence, the statistics tell us that the infants were responding to hue and not brightness differences in the checked patterns. This is most clearly seen in Wooten's Table 2 for my 5G-5B pairing, where differences in brightness were minimal under illuminant A (rank 11.5 out of 12) but the hue difference (rank 6 out of 12) elicited a percentage fixation of 75.

To provide a further test of preferences for checkerboards of different hues, I have observed the visual fixations of five males and five females 22 weeks old for the 10R-10G checkerboard paired with its control targets over four 5-second trials under illuminant C (*1*). The Y_C values of 19.63 and 19.68 listed in Wooten's Table 1 for 10R and 10G, respectively, represent the most similar brightness values possible for any of my checkerboards. In addition, the difference between the Y_C values of 10R and 10G is smaller than any brightness differences in any checkerboard under illuminant A in my original report. For these ten new subjects the 10R-10G checks elicited an average of 86.8 percent of total fixation (standard deviation, 6.77 percent), a percentage significantly higher than would be expected by chance ($t = 17.2$, d.f. 9, $P < .001$), and close to the average of 84.1 percent obtained for 10R-10G in my first study (*2*).

In short, when statistical or empirical controls are applied to brightness differences, the results indicate hue discrimination on the part of the infant. While this conclusion assumes that the luminosity function of infants 4 to 6 months old approximates that of the adult, it is not necessary that infants and adults have "precisely the same spectral sensitivity" in order to vary some range of brightness and hue differences and ask which determines infant preference behavior. The alternative of assuming widely divergent (and as yet unspecified) luminosity functions for infants and adults in order to account for infants' preferences for checkerboards on the basis of brightness rather than hue does not appear to be a reasonable or productive pursuit.

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Notes

1. The incandescent lighting was passed through a Roscolene No. 851 daylight blue, gelatin filter mounted between two pieces of clear Plexiglas 0.20 cm thick.
2. If the 10R-10G pairing under illuminant C is added to the 12 pairs in my first study and Wooten's ranking system is used, that pairing ranks as first in preference and thirteenth in brightness difference and ties with 10R-10G under Y_A to yield a rank of 2.5 in hue difference. The Kendall rank correlation coefficients, using all 13 pairings, are .813 for hue differences and preferences (highly reliable) and .309 for brightness differences and preferences (not statistically significant).
3. I thank P. C. Hughes and J. F. McNelis from the General Electric Applied Research Laboratory for providing technical assistance in approximating illuminant C.

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