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Maureene Selesky,[1] *Vina Spiehler,*[2] *R. H. Cravey,*[3] *and H. W. Elliot*[2]

Digoxin Concentrations in Fatal Cases

Analytical methods for the determination of digoxin in pharmaceutical and autopsy specimens using colorimetry [*1*], gas liquid chromatography [*2–4*], fluorometry [*1,5*], and polarography [*6*] have been reported. These classic methods, however, suffered from lack of sensitivity and specificity in the therapeutic (1.0 to 1.4 μg/litre) [*7*] and borderline toxic (2.0 μg/litre or greater) ranges, making detection of this drug possible only when large amounts remained unadsorbed in the stomach or excreted via the kidneys. The introduction of radioimmunoassay in 1969 by Smith et al [*7*] and Smith and Haber [*8*] made possible the detection of digoxin in postmortem biological fluids and tissue samples. Radioimmunoassay for the determination of serum digoxin is now the most extensively used radioisotope test in many nuclear laboratories and may be the most commonly requested drug assay by hospital physicians.

Determination of digoxin concentrations from extracts of postmortem tissue, urine, and serum samples by radioimmunoassay was reported by Brock [*9*] and Phillips [*10*]. Brock [*9*] used dichloromethane for the extraction of digoxin from tissues and reported that recovery of digoxin varied from 60 to 94% depending on the protein content of the original sample. Phillips [*10*] reported a dialysis method of extracting digoxin from postmortem blood. Karjalainen et al [*11*] reported postmortem tissue concentrations for patients who had been on maintenance digoxin therapy; their process employed extraction and radioimmunoassay techniques as described by Brock [*9*]. Iisala and Nuutila [*12*] reported postmortem serum digoxin concentrations in 3 suicide cases and Holt and Benstead [*13*], in 13 cases. Holt and Benstead [*13*] used tritiated water as an internal standard to correct for quenching caused by hemolysis. Moffat [*14*] reviewed 13 fatal poisonings in the United Kingdom involving suspected overdoses of cardiac glycosides. DiMaio et al [*15*] reported digoxin concentrations in postmortem blood, vitreous humor, and urine samples. Quench correction was accomplished by use of automatic external standardization in the liquid scintillation counter [*15,16*].

Fifteen postmortem coroners' samples of blood and tissue from persons with histories of having taken digoxin were analyzed. A recently developed radioimmunoassay test for digoxin employing dual isotope tracers (^{125}I and ^{57}Co) and an immunological "double antibody" separation technique was used. Use of a gamma emitter allowed direct assay of tissue homogenates and whole blood without the need to correct for quench.

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[1]Radioimmunoassay Center, Scientific Instruments Div., Beckman Instruments, Inc., Irvine, Calif. 92664.

[2]Department of Medical Pharmacology and Therapeutics, University of California, Irvine, Calif.

[3]Toxicology Laboratory, Office of the Sheriff-Coroner, Santa Ana, Calif.

Materials and Methods

Samples

Tissue samples were weighed and homogenized with 5% methanol-distilled water in a ratio of approximately 1 ml:1 g of tissue. A 100-μl aliquot of the homogenate, blood, serum, or urine was used for the assay.

Reagents

The digoxin determinations were made with reagents supplied by Beckman Instruments, Inc. The manufacturer's protocol was followed for performance of the tests. The assay involved the competition of the ^{125}I-labeled digoxin with the unlabeled digoxin contained in the serum for available binding sites on an antibody (referred to as the primary antibody). A secondary or precipitating antibody precipitated the primary antibody to which a portion of the labeled and unlabeled digoxin was bound. The precipitate containing the bound drug was separated by centrifugation and counted in a solid-state (gamma) scintillation counter.

Lyophilized ^{125}I-labeled digoxin, with ^{57}Co-solution monitor, was used. Iodine-125, attached to a functional group, was covalently bonded to the sugar moiety of the digoxin molecule. Cobalt-57 dichloride in solution was added to the ^{125}I-labeled digoxin as an internal standard.

Lyophilized digoxin antiserum (rabbit) to digoxin was used. Cross reactivity as reported by the manufacturer, relative to digoxin as 1.00, was digoxin, 1.0; digitoxin, 0.01; lanastoside C, 0.65; ouabain <0.001; spironolactone <0.001; testosterone <0.001; and progesterone <0.001. Serum albumin levels above 2% do not affect the results obtained with this reagent system. Lyophilized digoxin-precipitating antibody (goat anti-rabbit gamma globulin) was used as the second antibody. A water-soluble polymer was added as a precipitation accelerator.

Standards for digoxin ranging from 1.0 to 6.0 μg/litre were prepared gravimetrically by adding dry assayed digoxin in 5% methanol to defibrinated human plasma. Digoxin buffer, composed of digoxin-free human defibrinated plasma, was used for blanks (nonspecific binding) and zero concentration (maximum binding) as well as to dilute samples whose concentrations exceeded the limits of the standard curve.

Calculations

A Beckman Biogamma® equipped with the MB-1000 on-line data reduction system was used to quantitate, calculate, and record the assay results. The MB data system gave a ratio of the corrected standard and sample counts to the total count to obtain a "percent bound":

$$\%B = [(\text{sample counts—blank})/(\text{total counts—blank})]\ 100$$

The percent bound was plotted against the concentration of the standards. From the standard curve the sample concentrations were computed by linear interpolation and printed. The blank, standards, and sample counts were corrected using the ^{57}Co internal standard as follows:

$$\text{corrected counts} = (C_A - C_B)[(TC_A - C_A)/(TC_B - C_B)]$$

where

C_A = observed counts in ^{125}I channel,
C_B = observed counts in ^{57}Co above ^{125}I channel,
TC_A = average counts observed in the ^{125}I channel for the total count tube, and
TC_B = average counts observed in ^{57}Co above ^{125}I channel for the total count tube.

The counting windows were set up so that 5% of the ^{57}Co counts spilled over into the ^{125}I window.

Results

In the papers cited above, the use of ^{3}H-labeled digoxin required extractions to eliminate interference by tissue homogenates, bile, and hemoglobin pigments which cause color quenching (an effect in which light emitted by the fluor is reabsorbed by sample components and therefore not detected by the photomultiplier tube in the liquid scintillation counter). Synthesis of immunologically reactive, ^{125}I-labeled digoxin allowed replacement of the ^{3}H-labeled digoxin. Iodine-125 can be determined quantitatively in a simple solid-state scintillation (gamma) counter, thus providing a means for the direct assay of samples without extractions, preparation of liquid scintillation counting solutions, or time-consuming quench corrections.

Charcoal, as an agent to separate bound digoxin from unbound in the radioimmunoassay technique, has been replaced in this assay with the second antibody, which reacts immunologically with the digoxin antibody complex to form a protein superpolymer which is easily precipitated. Immunological precipitation, being specific for the antigen-antibody complex, eliminates the precipitation of unbound fraction along with the bound and the nonspecific binding of the digoxin to proteins rather than the antibody. This method of separation improves the precision of the assay, especially in the testing of tissue homogenates.

The use of an isotopic internal standard has been reported by Gotschlich [*17*] and by Gaze et al [*18*]. The ^{57}Co internal standard reduces the need for complete separation of the bound from the unbound antigen. After centrifugation and decantation, there is usually a varying amount of supernatant remaining trapped in the precipitate or on the walls of the tube. This supernatant will contain some unbound antigen. The usual procedure is to wash the precipitate one or more times to reduce the amount of unbound drug. However, this not only adds to assay time, it also increases the likelihood that some of the bound fraction is lost. With the use of the ^{57}Co as an internal monitor, washing and recentrifugation is eliminated. The amounts of supernatant, unbound ^{125}I-labeled digoxin, and ^{57}Co remaining in the tube are all proportional. Therefore, counts of the second isotope can be used to correct for the unbound digoxin remaining in the tube. In practice, this correction technique requires a gamma counter with two or more separate channels.

Case Histories

Ages of the deceased ranged from 3 days to 90 years (Table 1). In 10 of the 15 cases, elevated blood digoxin concentrations ranging from 3.5 to 30.0 μg/litre were found. Digoxin concentrations in Cases 1 through 4 appeared to be in the low to normal therapeutic range. In Case 3, death appeared to be due to alcohol intoxication. However, digoxin concentrations in the therapeutic range may be toxic to individuals with hypokalemia, hypercalcemia, hypomagnesemia, acid-base disturbances, increased adrenergic tone, hypothyroidism, hypoxemia, myocardial ischemia, or advanced heart disease [*19*].

Digoxin concentrations in Cases 5 through 7 exceeded the acceptable therapeutic range. However, Iisalo and Nuutila [*12*] have reported that blood digoxin concentrations

TABLE 1—*Blood concentrations of digoxin in cases studied.*

Case	Age, years	Sex	Daily Dosage, mg	Estimated Time Elapsed Before Sample Obtained, h	Average Blood Concentration, ng/ml	Remarks
1	90	f	0.125	1.5	0.7	no autopsy
2	72	m	unknown	48	0.74	no autopsy
3	51	m	0.25	36	1.07	death caused by alcohol intoxication
4	31	m	unknown	17	1.29	...
5	65	m	0.25	24	2.20	jumped from bridge
6	49	f	0.25	4	2.59	...
7	74	m	0.125	2	2.63	no autopsy
8	43	f	0.25	7	3.53	no autopsy
9	83	f	unknown	14	3.57	no autopsy
10	85	f	unknown	15	5.29	no autopsy
11	74	m	unknown	8	5.64	no autopsy
12	38	f	0.25	46	7.25	propoxyphene, fatal
13	2	f	0.04 mg/kg	8	6.44	accidental amitriptyline overdose
14	64	f	100 × 0.25 mg	4	15.25	suicide
15	neonate	m	4.0	6	30.3	...

increase with elapsed time between death and autopsy. The 24-h period between death and obtaining the blood sample in Case 5 may have contributed to the elevated concentration of digoxin.

Digoxin concentrations in Cases 8 through 15 were considerably above therapeutic concentrations. In Cases 14 and 15, an elevated dose was reportedly administered. Smith and Willerson [*20*] report that recovery is possible even after serum digoxin levels of up to 20 μg/litre, when the patient is managed with atropine, antiarrhythmic drugs, and electrocardiographic monitoring. Factors which decrease an individual's sensitivity to digoxin are hyperkalemia, hyperthyroidism, quinidine, procainamide, and propanolol [*19*]. Detailed histories of cases are presented below.

Case 1

An autopsy was performed on a 90-year-old female within 2 h after her death, which was determined to be due to a myocardial infarction. Lanoxin®, one 0.125-mg tablet daily, had been prescribed for her. Postmortem toxicology revealed a blood digoxin concentration of 0.70 μg/litre.

Case 2

An autopsy was performed 2 days after the death of a 72-year-old male who weighed 250 lb (113 kg). The deceased has been taking Lanoxin and Lasix® (dosage unknown). The cause of death was determined to be cardiomegaly resulting from congestive heart failure and arteriosclerotic heart disease. Toxicological examination of the blood revealed a low digoxin concentration of 0.74 μg/litre.

Case 3

Doriden®, Empirin® Compound with codeine phosphate #3, and Tylenol® with codeine #4 had been prescribed for a 51-year-old male approximately 10 days before his death. The deceased was also taking 0.25 mg digoxin daily. All the bottles, except for the digoxin, were found empty. The cause of death was determined to be due to ethanol and diazepam intoxication. The autopsy was performed two days after his death. Toxicological findings revealed ethanol concentrations of 400 mg/100 ml in the blood and 350 mg/g in the brain. Diazepam concentrations of 0.05 mg/100 ml in the blood and 0.35 mg/100 g in the liver were also found. A digoxin concentration of 1.07 μg/litre in the blood was found.

Case 4

A 81-year-old male was treated with digoxin (dosage unknown). The cause of death was determined to be due to thrombosis, right coronary artery, resulting from arteriosclerotic heart disease. Other significant pathology included myocardial fibrosis and hypertensive cardiovascular disease. Toxicological examination 17 h later revealed a blood digoxin concentration of 1.29 μg/litre.

Case 5

A 65-year-old male suffered fatal injuries as a result of jumping from a bridge. He had been treated with 0.25 mg digoxin daily and unknown dosages of Artane®, Mellaril®, and Thorazine®. Autopsy findings the following day revealed a blood digoxin concentration of 2.2 μg/litre.

Case 6

A 49-year-old female who was being treated with 0.25 mg digoxin daily died at her home. The cause of death was certified by the hospital physician as myocardial infarction resulting from arteriosclerotic heart disease. Blood obtained within 4 h of her death revealed a digoxin concentration of 2.59 μg/litre.

Case 7

A 74-year-old male who had been treated with 0.125 mg digoxin daily collapsed suddenly while playing shuffleboard. He was pronounced dead on arrival at the local hospital. The cause of death was certified as ventricular tachycardia resulting from congestive heart failure. Blood drawn the same day by a mortician revealed a digoxin concentration of 2.63 μg/litre.

Case 8

A 43-year-old female who weighed 100 lbs (45 kg) had been treated with Tedral-25® (1 or 2 tablets every 4 h when needed), Lasix (20 mg as needed), and digoxin (0.25 mg daily). An autopsy was performed several hours after her death. Toxicological findings revealed a digoxin blood concentration of 3.53 μg/litre. Blood, liver, and stomach contents were screened for other drugs; none were found. The cause of death was given as cor pulmonale with congestive heart failure resulting from pulmonary emphysema.

Case 9

An 83-year-old female's death was registered by her family physican as being caused by arteriosclerotic heart disease. Blood drawn by a mortician 3 h after death revealed a digoxin concentration of 3.57 μg/litre.

Case 10

The death certificate of an 85-year-old female was signed by her physician and no autopsy was performed. She had been administered digoxin daily, dosage unknown. Blood drawn the following morning by the mortician revealed a blood digoxin concentration of 5.29 μg/litre.

Case 11

An autopsy performed on a 74-year-old male the day after his death revealed a blood digoxin concentration of 5.64 μg/litre. The cause of death was certified as occlusion, right and left coronary arteries, resulting from arteriosclerotic cardiovascular disease. No history was obtained as to digoxin treatment.

Case 12

A 38-year-old female psychiatric patient with a history of suicide attempts died of an overdose of propoxyphene and digoxin. Specimens obtained at autopsy 46 h after death were analyzed. Propoxyphene was found present in the blood in the concentrations of 1.0 mg/100 ml and in the liver in concentrations of 17.8 mg/100 ml. Approximately 1 mg propoxyphene was found in the total stomach contents. Digoxin was found in the blood in the concentration of 7.25 μg/litre. Lanoxin, 0.25 mg daily, had been prescribed. At autopsy, the lungs showed chronic fibrous emphysema, basilar congestion, and patchy atelectasis. The heart appeared hypertensive with chronic pericarditis.

Case 13

A two-year-old female was admitted to hospital at 7 p.m. The child was suspected of taking an unknown amount of amitriptyline from a relative's prescription. Digoxin was given intramuscularly, 0.04 mg/kg, at 3 a.m. the following morning. Half the dose was given initially, and one fourth the remaining dose was given every 6 min until the injection was completed. Since the child did not respond to digoxin after 2 h, 0.1 to 0.2 mg Inderal® was given intermittently over 6 min. The child died at 4:45 a.m. the following morning and an autopsy was performed at 1:20 p.m. Amitriptyline, 0.03 mg/100 g, was found in the liver by gas liquid chromatography. Nortriptyline was present but not quantitated. The postmortem blood digoxin concentration was 6.44 μg/litre.

Case 14

A 64-year-old female weighing 160 lbs (72 kg) told her husband at breakfast that she had taken 100 digoxin tablets (0.25 mg) at 1:00 a.m. and had vomited at 4:00 a.m. She had attempted suicide previously. She was admitted to the hospital at 9:00 a.m., lucid and coherent. She vomited again at admission. A serum sample taken at 9:00 a.m. contained 14.4 μg/litre digoxin; plasma (lithium, heparin preserved) contained 14.8 μg/litre digoxin; whole blood, 15.17 μg/litre; and urine, 14.1 μg/litre (Table 2). The woman suffered cardiopulmonary arrest 40 min after admission and was given epinephrine and

TABLE 2—*Toxicology results from Case 14. Antemortem blood samples were taken 9 h after patient claimed to have ingested 25 mg of digoxin. The patient died 9 h, 40 min after ingestion. Interval between death and the time the postmortem blood sample was taken was 6 h, 35 min.*

Sample	Preservative	Date and Time	Digoxin Concentration, ng/ml
Serum	none	8/24/75; 9 a.m.	14.42
Urine	none	8/24/75; 9 a.m.	14.1
Plasma	lithium, heparin	8/24/75; 9 a.m.	14.80
Plasma	EDTA	8/24/75; 9 a.m.	17.65
Blood	none	8/24/75; 9 a.m.	15.17
Postmortem blood	potassium oxalate	8/24/75; 3:35 p.m.	15.05

lidocaine. She died at 10:50 a.m. An autopsy was performed at 3:30 p.m. on the same day. Postmortem blood (potassium oxalate preserved) contained 15.05 μg/litre digoxin.

Pathology findings included moderate arteriosclerosis. The lungs showed a moderate edema and congestion. The liver was congested.

Case 15

A 3-day-old, 2.2-kg male neonate with congenital heart disease was given a blood exchange and administered 3.0 mg digoxin at 12:45 p.m. and 1.0 mg at 3:00 p.m. The recommended total "digitalizing dose" for neonates is 0.04 mg/kg [*21*] or 0.088 mg in 24 h for a 2.2-kg child. The digoxin concentration on blood drawn at 5:45 p.m. was reported as 7.3 μg/litre. The neonate died at 6:10 p.m. Digoxin values on postmortem blood and tissue samples obtained at autopsy 6 h after death are given in Table 3.

TABLE 3—*Digoxin values on postmortem blood and tissue samples for Case 15. The three-day-old infant received 3 mg digoxin initially and 1 mg 2 h later. Antemortem blood samples taken 3 h after last dose were reported as 7.3 ng/ml. Death occurred 3 h, 10 min after last dose. Postmortem blood samples taken 4 h after death were 30.3 ng/ml.*

Sample	Digoxin Concentration	Tissue Digoxin to Blood Digoxin Ratio	
		Case 15	Steentoft's Case [*1*]
Blood	3.03 ng/ml	1.0	1.0
Brain	0.86 ng/g	0.0284	...
Liver	35.32 ng/g	1.165	1.53
Kidney	130 ng/g	4.29	4.53
Spleen	51.63 ng/g	1.70	...
Lung	45.33 ng/g	1.49	...
Urinary bladder	>40 ng/g	>1	2.53

Discussion

Iisalo and Nuutila [*12*] and Karjalainen et al [*11*] reported an increase in blood digoxin concentrations with time after death. In Case 14, no increase in the blood digoxin concentration was observed between the antemortem samples (15.7 μg/litre) and samples obtained at autopsy 6½ h later (15.05 μg/litre). The elevated digoxin values seen in Cases 5, 10, 12, and 15 may be due to the interval between death and sampling.

Steentoft [*1*] reported a case similar to that of our Case 15. A neonate with congenital heart disease was administered 0.1 mg digoxin intravenously three times daily for 2 days and subsequently 0.7 mg digoxin 1 h prior to death. Tissue and blood samples were assayed fluorometrically for digoxin [*1*]. The calculated tissue to blood ratio for values reported by Steentoft [*1*] and those performed on our neonate were in agreement (Table 3). Tissue digoxin concentrations reported above and by Steenthoft suggest that in the neonate digoxin is concentrated in the kidney and urine but not to the extent reported by Doherty et al [*22*] in the adult. It also may be found in the lungs, spleen, and liver in concentrations equal to or slightly greater than blood concentrations. At 30 μg/litre blood digoxin concentrations in the neonate brain digoxin concentrations are approximately 3% of blood concentrations. This concentration was easily assayed by radioimmunoassay.

In Case 14 antemortem serum, plasma, and urine concentrations were 95% of whole blood values. No difference in digoxin concentrations between serum and plasma with heparin anticoagulant was observed; however, plasma samples with ethylenediaminetetraacetic acid (EDTA) gave a value 22% higher than serum digoxin concentrations. Further investigation of anticoagulants used with this digoxin system is now being conducted.

Conclusions

Improvements in digoxin radioimmunoassay methods have made possible the direct assay of postmortem tissue homogenates and whole blood samples. The ability to use ^{125}I instead of ^{3}H as the digoxin tracer has eliminated the need for extractions as well as quench corrections. Also, improvements seen with immunological separation assure the investigator of increased precision in his digoxin values.

Iisalo and Nuutila [*12*] and Karjalainen et al [*11*] reported an increase in blood digoxin concentrations with time after death. Doherty et al [*22*] suggest that after death a new equilibrium between the blood and tissues is established resulting in higher digoxin concentrations in heart blood. While this may be true, such an increase was not noted in our Case 14, where we had occasion to measure the antemortem samples and the samples collected at autopsy 6½ h later.

Holt and Benstead [*13*] have suggested that in those cases in which digoxin has been administered before death that it may be helpful to know the blood digoxin concentrations postmortem, especially in those cases where cardiovascular lesions are absent and no morbid anatomical cause of death is found. In the limited number of cases we have studied, it appears important for the toxicologist to determine the postmortem blood digoxin concentration in all cases where digoxin has been prescribed. Considering the relative potency of digoxin (therapeutic index), its importance may have previously been ignored because of the lack of adequate methods for detection and estimation.

References

[*1*] Steentoft, A., "Fatal Digitalis Poisoning," *Acta Pharmacologica et Toxicologica,* Vol. 32, 1973, pp. 353–357.

[*2*] Kibbe, A. H. and Araujo, O. E., "Rapid Quantitative GLC Analysis of Digoxin," *Journal of Pharmaceutical Sciences,* Vol. 62, No. 10, 1973, pp. 1703–1704.

[*3*] Watson, E. and Kalmon, S. M., "Assay of Digoxin in Plasma by Gas Chromatography," *Journal of Chromatography,* Vol. 56, 1971, pp. 209–218.

[*4*] Tan, L., "An Improved Method for the Gas Chromatographic Identification of Digitalis Cardenolides," *Journal of Chromatography,* Vol. 45, 1969, p. 68.

[*5*] Jellife, R. W. and Stephenson, R. G., "A Fluorometric Determination of Myocardial Digoxin at Autopsy," *American Journal of Clinical Pathology,* Vol. 51, 1969, pp. 347–357.

[*6*] Kadish, K. M. and Spiehler, V. R., "Differential Pulse Polarographic Determination of Digoxin and Digitoxin," *Analytical Chemistry,* Vol. 47, No. 9, 1975, pp. 1714–1716.

[7] Smith, T. W., Butler, V. P., and Haber, E., "Determination of Therapeutic and Toxic Serum Digoxin Concentrations by Radioimmunoassay," *New England Journal of Medicine,* Vol. 281, 1969, pp. 1212–1216.

[8] Smith, T. W. and Haber, E., "Digoxin Intoxication. The Relationship of Clinical Presentation of Serum Digoxin Concentration," *Journal of Clinical Investigation,* Vol. 49, 1970, pp. 2377–2386.

[9] Brock, A., "A Radioimmunoassay for Digoxin in Serum, Urine and Myocardial Tissue," *Acta Pharmacologica et Toxicologica,* Vol. 34, 1974, pp. 198–204.

[10] Phillips, A. P., "A Radioimmunoassay Technique for Digoxin in Postmortem Blood," *Journal of Forensic Sciences,* Vol. 19, No. 4, 1974, pp. 900–912.

[11] Karjalainen, J., Ojala, K., and Reissel, P., "Tissue Concentrations of Digoxin in an Autopsy Material," *Acta Pharmacologica et Toxicologica,* Vol. 34, 1974, pp. 385–390.

[12] Iisalo, E. and Nuutila, M., "Myocardial Digoxin Concentrations in Fatal Intoxications," *Lancet,* 3 Feb. 1973, p. 257.

[13] Holt, D. W. and Benstead, J. G., "Post-mortem Assay of Digoxin by Radioimmunoassay," *Journal of Clinical Pathology,* Vol. 28, 1975, pp. 483–486.

[14] Moffat, A. C., "Interpretation of Post-mortem Serum Levels of Cardiac Glycosides After Suspected Overdosage," *Acta Pharmacologica et Toxicologica,* Vol. 35, 1974, pp. 386–394.

[15] DiMaio, V. J. M., Garriott, J. C., and Putnam, R., "Digoxin Concentrations in Post-mortem Specimens After Overdose and Therapeutic Use," *Journal of Forensic Sciences,* Vol. 20, No. 2, 1975, pp. 340–347.

[16] Hellman, E. Z., Spiehler, V. R., and Holland, S., "Elimination of Error Caused by Hemolysis and Bilirubin-Induced Color Quenching in Clinical Radioimmunoassays," *Clinical Chemistry,* Vol. 20, No. 9, 1974, pp. 1187–1193.

[17] Gotschlich, E. C., "A Simplication of the Radioactive Antigen Binding Test by Double Label Technique," *Journal of Immunology,* Vol. 107, No. 3, 1971, pp. 910–911.

[18] Hunter, W. M., "Radioimmunoassay," Chapter 17 of *Handbook of Experimental Immunology,* G. Weir, Ed., Blackwell Scientific, 2nd printing 1969, p. 17.14.

[19] Shapiro, B., Kollman, G., and Heine, W., "Pitfalls in the Application of Digoxin Determinations," *Seminars in Nuclear Medicine,* Vol. 5, No. 3, 1975, pp. 205–217.

[20] Smith, T. W. and Willerson, J. T., "Suicidal and Accidental Digoxin Ingestion," *Circulation,* Vol. 44, 1971, pp. 29–36.

[21] Neill, C. A., "The Use of Digitalis in Infants and Children," *Progress in Cardiovascular Diseases,* Vol. 7, No. 5, 1965, pp. 399–416.

[22] Doherty, J. E., Perkins, W. H., and Flanagan, W. J., "Distribution and Concentration of Tritiated Digoxin in Human Tissues," *Annals of Internal Medicine,* Vol. 6, 1967, pp. 116–124.

Radioimmunoassay Center
Scientific Instruments Div.
Beckman Instruments, Inc.
Campus Drive at Jamboree Blvd.
Irvine, Calif. 92664