Elimination of Error Caused by Hemolysis and Bilirubin-induced Color Quenching in Clinical Radioimmunoassays

Edith Zak Helman, Vina Splehler, and Susan Holland

In radioimmunoassays and radioassays for various constituents of serum, color quenching is a source of error when liquid-scintillation methods are used in samples that contain hemolyzed blood and samples from jaundiced patients. A quench correction can be made by using the External Standard Channels Ratio method and normalizing the ratios, or, alternatively, by chemical decolorization with tetrathymethylammonium hydroxide, hydrogen peroxide, and ascorbic acid.

Most compounds of clinical interest used in radioimmunoassays are labeled with $^{125}$I and hence their radioactivity is best measured by solid-crystal gamma counters. However, some steroid hormones and drugs are labeled with $^3$H or $^{14}$C and because of the low energy of the beta radiation emitted as these radionuclides disintegrate, they are best counted by liquid scintillation methods in which the labeled substance is intimately mixed with a liquid scintillator.

Isotopes that emit gamma or x-rays or both (e.g., $^{125}$I, $^{57}$Co, $^{51}$Cr, and $^{59}$Fe) also emit Auger electrons or beta particles or both during their radioactive decay, and these can also be counted by conventional liquid-scintillation methods—i.e., after mixing the labeled substance with a liquid scintillator (1).

However, anything that interferes with the scintillation process prevents light from reaching the photomultiplier tube and thus decreases both the number of recorded counts and the apparent energy of the counts. This phenomenon is called quench. Impurities in the “cocktail” material cause quenching. Quenching can also result from insufficient amounts of fluor or solvent in the cocktail or from too large a sample volume. Color quenching occurs when light-absorbing colored substances present in the sample absorb the light emitted by the scintillator before it can escape the vial and reach the photomultiplier tube.

In biological samples encountered in the clinical laboratory, color quenching is mainly attributable to light absorbance by hemoglobin and bilirubin in the specimen. These compounds absorb highly in the same region in which fluoros emit light and in which the photomultiplier tube detector is most sensitive (Figure 1).

The net result is that the count per minute (cpm) registered by the liquid scintillation system is decreased in relation to the activity of the sample, designated as disintegrations per minute (dpm). Hence, the efficiency of counting, as expressed by the formula (cpm/dpm) x 100 = percent efficiency, is diminished. This decrease in counts attributable to color quenching by bilirubin and hemoglobin can lead to

---

Radioimmunoassay Center, SID Applications Research, Beckman Instruments, Inc., Campus Drive at Jamboree Blvd., Irvine, Calif. 92664.

Received April 15, 1974; accepted June 4, 1974.

Fig. 1. Absorption spectra of hemoglobin and bilirubin compared to the wavelength dependence of the quantum efficiency of the liquid-scintillation counter photomultiplier tube (PMT). Quantum efficiency is not drawn to scale. The absorption maxima of the colored compounds coincide with the sensitive region of the PMT, resulting in significant interference.
erroneous results in radioimmunoassays, if not corrected (2).

Cerco and Elloso (3) reported interference by hemoglobin in digoxin radioimmunoassays when a toluene-based cocktail was used. Wien and Kumar (4) found that use of a dioxane cocktail similar to Bray's alleviated the problem of interference from hemoglobin. We found that this was because the hemoglobin is precipitated. However, Bray's cocktail also has disadvantages—such as chemiluminescence, protein precipitation, and low efficiencies—that detract from its use in the clinical laboratory; furthermore, color-quenching by bilirubin is still a problem with Bray's cocktail.

The object of this study was to determine the extent of the quench problem in clinical radioimmunoassays and radioassays and to correct this quenching by instrumental and chemical means.

Materials and Instrumentation

The following products were used in this study: Hycel Hemoglobin Control, Hycel Inc., Houston, Texas 77055; Digox-El, A. R. Smith, P.O. Box 5400, Terminal Annex, Los Angeles, Calif. 90017; Hyland II Abnormal Sera, Hyland Division, Travenol Laboratories, Costa Mesa, Calif. 92626.

The following Beckman products were also used: Ready-Solv VI (liquid-scintillation mixture), P/N 566436; Value Vials (glass liquid-scintillation counting vials), P/N 161650; Bio-Vials (polypropylene gamma-counting vials), P/N 566353; Poly-Q-II Vials (polypropylene liquid-scintillation counting vials), P/N 566350; Poly-Q Vial Cap with hole, P/N 58225; Digoxin Radioimmunoassay Kit, P/N 566132; T3 Uptake Kit, P/N 566148; Tetramethylammonium Hydroxide, 1.0 mol/liter, P/N 184949; ascorbic acid solution, 150 g/liter, P/N 188036; Liquid Scintillation Counters LS-100C, P/N 166125U and LS-250, P/N 169605U; Gamma Counter-Beckman Biogamma, P/N 167776.

Experimental Procedures

Several experiments were designed to determine the extent of the problem of quenching in clinical radioimmunoassays, devise a simple instrumental method of quench correction by using normalizing ratios, determine whether it is necessary to run both hemoglobin and bilirubin quench curves, and set up a method for daily quench controls. Finally, quench correction by chemical decolorization was studied. Each experiment is reported separately below. The conclusion at the end of the paper summarizes the relative values of the instrumental and chemical methods of correcting for quench correction.

Determination of Extent of Quench Problem

Methods. Hemoglobin quench standards were prepared that simulated the end results of a digoxin radioimmunoassay. These contained constant volumes of liquid-scintillation cocktail, buffer, tritiated water, and serum containing increasing amounts of hemoglobin. Counting efficiencies were plotted vs. hemoglobin concentrations of the hemolyzed specimens (Figure 2).

Bilirubin quench standards were prepared by adding to liquid-scintillation cocktail constant volumes of serum, buffer, and 125I as used in a triiodothyronine uptake assay. These standards contained increasing amounts of bilirubin dissolved directly in the cocktail. Efficiencies were plotted vs. bilirubin concentration as related to the original serum sample (Figure 3).

Results and conclusion. Figures 2 and 3 show that even low concentrations of hemoglobin and bilirubin can affect the efficiencies of counting and, therefore, correction for color quenching must be made if correct results are to be obtained in competitive binding assays. The frequently recommended method of in-
ternal standardization is tedious and subject to pipetting errors and does not lend itself to larger numbers of assays or computerization. Therefore, two alternative methods of quench correction were evaluated—instrumental methods by External Standard Channels Ratio and Chemical Decolorization.

Use of External Standard Channels Ratio Method for Quench Correction

The External Standard Channels Ratio (ESCR) involves a widely used instrumental method that can be used to detect and measure quenching in a sample (5). Some instruments are equipped to do this automatically, by subjecting each sample to radiation from an external radioisotope. The system then measures the response of the sample and scintillation cocktail to that radiation in two counting windows, calculates a ratio from these measurements, and prints that ratio (External Standard Channels Ratio number) along with the counts for the sample itself. The ESCR number decreases as the counting efficiency decreases because of quench.

Traditionally, a quench series has been used to determine the percent efficiency of counting. This in turn can be used to determine absolute activity of the sample. Percent efficiency is defined as the sample cpm divided by the dpm of a standard times 100 [%E = (cpm/dpm) × 100]. Percent efficiency of sample counting is determined from a plot of ESCR vs. percent efficiency. The sample count per minute is divided by the percent efficiency/100 for the sample to give sample disintegrations per minute. To compare the counts of the sample to the standard curve used in the assay, the percent efficiency at which the standards were counted is determined from the graph of ESCR vs. percent efficiency. The sample dpm is multiplied by the standards efficiency/100 to give the corrected counts (cpm).

In the clinical laboratory, the absolute activity of a radioisotope having a short half-life, such as \(^{125}\)I, is often not known; therefore, substitution of normalizing ratios instead of percent efficiency can be useful in correcting for quench. The use of normalizing ratios instead of percent efficiency is described below.

Comparison of Percent Efficiency vs. Normalizing Ratios to Correct for Quench

Methods. A hemoglobin quench series for a digoxin assay was set up as follows:

To eight glass counting vials were added 0.2 ml of serum, 0.1 ml of tritiated water of known dpm, and hemoglobin control and buffer as shown in Table 1.

After adding 10 ml scintillation fluid, capping and vortex-mixing, each sample was placed in the liquid-scintillation counter to obtain sample counts and ESCR numbers. The ESCR numbers were then plotted against percent efficiency and normalizing ratio.

The normalizing ratio for each standard, plotted on the right-hand axis of Figure 4, was determined by dividing the count of each sample by the count of the sample that contained no hemoglobin (Table 1).

**Results and conclusion.** Fewer arithmetic steps were involved when normalizing ratios were used to correct the counts for a quenched sample. For example, a sample having an ESCR number of 0.540 (Figure 4) was counted at 30% efficiency. If the cpm of this sample was 1000, the actual activity of the sample (dpm) would be:

\[
dpm = \frac{\text{cpm}}{\% \text{ efficiency/100}} = \frac{1000}{0.3} = 3333
\]

Because the standards were counted at 40% efficiency (determined by calculating the efficiency of the least quenched standard) the sample with no hemoglobin should have had a cpm of 1333 (i.e., 3333 × 0.40).

In using normalizing ratios, one simply uses the right-hand axis to determine that a sample having an ESCR of 0.540 has a normalizing ratio of 0.75. Dividing the 1000 cpm by 0.75 gives the corrected value of 1333. Thus, use of normalizing ratio simplifies calculations and obviates the need to know the absolute activity (dpm) of the isotope in calculating percent efficiency.

Although the previous data were obtained on hemoglobin standards, it was desirable to determine whether it is necessary to run separate hemoglobin and bilirubin quench curves.

**Table 1. Preparation of Samples for Hemoglobin Quench Series—Digoxin Assay**

<table>
<thead>
<tr>
<th>Sample no.</th>
<th>Kit buffer ml</th>
<th>Hycel Hb control 50 fold dilution</th>
<th>cpm</th>
<th>ESCR*</th>
<th>N.R. a</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.3</td>
<td>0</td>
<td>11310</td>
<td>.616</td>
<td>1.00</td>
</tr>
<tr>
<td>2</td>
<td>1.3</td>
<td>0.05</td>
<td>10501</td>
<td>.590</td>
<td>.978</td>
</tr>
<tr>
<td>3</td>
<td>1.2</td>
<td>0.1</td>
<td>9722</td>
<td>.570</td>
<td>.860</td>
</tr>
<tr>
<td>4</td>
<td>1.0</td>
<td>0.3</td>
<td>7441</td>
<td>.505</td>
<td>.658</td>
</tr>
<tr>
<td>5</td>
<td>0.9</td>
<td>0.4</td>
<td>5459</td>
<td>.433</td>
<td>.483</td>
</tr>
<tr>
<td>6</td>
<td>0.7</td>
<td>0.6</td>
<td>4736</td>
<td>.405</td>
<td>.418</td>
</tr>
<tr>
<td>7</td>
<td>0.5</td>
<td>0.8</td>
<td>3892</td>
<td>.352</td>
<td>.344</td>
</tr>
<tr>
<td>8</td>
<td>0.3</td>
<td>1.0</td>
<td>2992</td>
<td>.310</td>
<td>.265</td>
</tr>
</tbody>
</table>

* External Standard Channels Ratio number.

a Normalizing ratio.
Comparison of Hemoglobin vs. Bilirubin Quench Curves

Methods. Hemoglobin quench series was prepared similar to Table 1 for a triiodothyronine uptake radioassay as follows: to each glass vial were added 0.3 ml serum, 0.1 ml ¹²⁵I solution containing about 0.02 μCi activity (44 000 dpm), 0–0.3 ml of a 10-fold dilution of Hycel hemoglobin control, and 0.3–0 ml of buffer to equalize the volumes.

After adding 15 ml of scintillation cocktail to each vial, capping and vortex-mixing, the vial was counted. From the counts, normalizing ratios were calculated and then were plotted vs. ESCR numbers (Figure 5).

The data for the bilirubin quench curve were obtained as above except that instead of hemoglobin, 0–0.6 ml of a 15 mg/dl stock solution of bilirubin in toluene was added to the cocktail. The data are plotted in Figure 5.

Results and conclusion. Figure 5 shows that, for this assay, the hemoglobin and bilirubin quench curves are superimposable. Because it is easier to prepare quench curves by using hemoglobin, only one set of daily quench controls (see below) is used to correct for both hemolysis and jaundice in this assay. This was also found to be true for digoxin assays.

It is not necessary to run a complete quench series daily. The complete quench series described above is recommended when first setting up a new assay, to identify the linear portion of the quench curve,¹ to choose the best concentrations of quenching agent for daily quench controls, and to determine if the quench curve for hemoglobin is valid for bilirubin. Once this is obtained, use only daily quench controls.

¹ Linear interpolation is most accurate on the linear portion of the curve; therefore, grossly hemolyzed or jaundiced samples must be decolorized or rejected.

Table 2. Preparation of Samples for Daily Quench Controls—Digoxin Assay

<table>
<thead>
<tr>
<th>Tube no.</th>
<th>Hb (mg/dl)</th>
<th>Hycel low-Hb control (μl)</th>
<th>cpm</th>
<th>ESCR</th>
<th>N.R.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>0</td>
<td>2239</td>
<td>0.558</td>
<td>1.00</td>
</tr>
<tr>
<td>2</td>
<td>100</td>
<td>50</td>
<td>1843</td>
<td>0.500</td>
<td>0.82</td>
</tr>
<tr>
<td>3</td>
<td>200</td>
<td>100</td>
<td>1765</td>
<td>0.477</td>
<td>0.78</td>
</tr>
<tr>
<td>4</td>
<td>400</td>
<td>200</td>
<td>1232</td>
<td>0.382</td>
<td>0.55</td>
</tr>
</tbody>
</table>

Preparation of Daily Quench Controls for Typical Assays

Digoxin assay—daily controls. Four milliliters of reconstituted digoxin control were diluted with 12 ml of glycoside free serum from the digoxin kit or with digoxin-free pooled serum. Four milliliters of the fourfold dilution were placed in each of the four tubes and 0 to 250 μl of hemoglobin control was added to the four tubes (Table 2). The tubes were capped and stored at 4 °C for one week.

Controls were run in duplicate. The recovery of digoxin in tube 1, which contains no hemoglobin, should be consistent with the manufacturer's stated value. From the counts obtained from the controls, tubes 1–4 (Table 2), normalizing ratios were calculated and plotted vs. ESCR ratios (Figure 6).

Samples from patients were run and corrected by relating ESCR to the corresponding normalizing ratio and dividing counts for patients by this ratio.

Using the ESCR number of the quenched sample as read from the printout, we obtained the corresponding normalizing ratio from the plot in Figure 6. Quenched patient sample counts were corrected by dividing the counts per minute by the appropriate normalizing ratio (Table 3).
If the counts had not been corrected, the digoxin values would have been 2.0 and 1.8 mg/ml digoxin, a 32% and 28% error, respectively.

Triiodothyronine uptake assay—daily controls. Control serum of known uptake values were supplemented with hemoglobin so as to contain 0, 100, 200, and 400 mg of hemoglobin per deciliter, and were run in the same way as patients' samples for daily quench controls. The normalizing ratios were plotted vs. ESCR from the printout.

Additional samples containing various concentrations of hemoglobin and bilirubin were run with the quench controls and the corrected counts were obtained by dividing the counts per minute by the appropriate normalizing ratio. The corrected and uncorrected uptake values were then compared to the expected uptake values obtained by counting the samples in a gamma counter, which is not subject to interference by quench (Table 4).

The corrected uptake results agreed with the expected results, whereas the uncorrected results would have been completely unacceptable.

Conclusion

A full series of quench standards, with use of hemoglobin and bilirubin, should be run when setting up a new assay, to determine the contribution to quench interference in that assay from assay reagents, hemoglobin, and bilirubin. The most nearly linear portion of the quench curve is established. Samples falling in this linear region can be corrected arithmetically for quench by using the ESCR and normalizing ratios determined from daily quench controls. The bilirubin and hemoglobin curves (ESCR vs. normalizing ratio or percent efficiency) should be plotted on the same graph, to determine whether separate or single daily quench controls must be used to correct for the two different quench agents. Once established, the full curve need not be run routinely; only daily checks need to be run.

Daily quench controls are used to check ESCR and normalizing ratio relationship. At least four controls are required: a control with no added quenching agent and the same control with added hemoglobin or bilirubin to correspond to the top, bottom and middle of the linear portion of the quench curve. These controls are included in each sample run.

The data from the plot of the ESCR and normalizing ratios can be used to correct the counts of the quenched samples for that run.

Evaluation of Quench Correction by Chemical Decolorization

Although the instrumental methods just described offer a practical way to correct for color quenching owing to hemoglobin and bilirubin in clinical radioimmunoassays and radioassays, some clinical laboratories may prefer to eliminate quenching by decolorizing the samples. The goal of chemical decolorization is to convert the porphyrin or unsaturated structure to a colorless form that will not interfere in liquid-scintillation counting.

Several techniques have been reported in the literature for eliminating interference caused by color including decolorization with hypochlorite bleach, ben-

<table>
<thead>
<tr>
<th>Sample</th>
<th>Quenching agent, mg Hb/dl</th>
<th>Uncorrected, cpm</th>
<th>ESCR</th>
<th>N.R.</th>
<th>Corrected, cpm</th>
<th>T-3 uptake</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>300</td>
<td>45320</td>
<td>0.283</td>
<td>0.66</td>
<td>69934</td>
<td>0.62</td>
</tr>
<tr>
<td>12</td>
<td>150</td>
<td>56299</td>
<td>0.406</td>
<td>0.80</td>
<td>70373</td>
<td>0.77</td>
</tr>
<tr>
<td>18</td>
<td>7.6</td>
<td>48683</td>
<td>0.385</td>
<td>0.77</td>
<td>63224</td>
<td>0.67</td>
</tr>
</tbody>
</table>

* Duplicate samples run on Biogamma.

Table 3. Correction of Hemoglobin-Quenched Sample Counts in Two Patients' Digoxin Assays, by Use of Normalizing Ratios

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>2110</td>
<td>.536</td>
<td>.93</td>
<td>2628</td>
<td>1.55</td>
<td>2.0</td>
</tr>
<tr>
<td>2159</td>
<td>.536</td>
<td>.93</td>
<td>2321</td>
<td>1.40</td>
<td>1.8</td>
</tr>
</tbody>
</table>

Table 4. Correction of Hemoglobin Quenched Sample Counts in T-3 Uptake Assay by Use of Normalizing Ratios
zoyl peroxide, or sodium borohydride (6, 7), precipitation with trichloroacetic acid (8), adsorption on charcoal (9), or treatment with hydrogen peroxide plus ascorbic acid or stannous chloride (10) or sodium hydroxide followed by hydrogen peroxide (11).

Each of these methods has drawbacks such as chemiluminescence, excessive quenching, long periods for decolorizing, or laborious steps that make the procedure impractical for routine clinical use. Furthermore, many of these methods eliminate the color caused by hemoglobin but not that caused by bilirubin. It was desirable to find a procedure that would decolorize both hemolyzed and bilirubin-containing samples and yet not decrease counting efficiency or have the above disadvantages.

Experiments were set up to determine the reagents necessary to decolorize samples from different types of radioimmunoassays and radioassays, and recovery experiments were performed.

**Materials and methods.** The effect of using different decolorizing methods was evaluated on a digoxin assay as an example of a tritiated analyte, and on a triiodothyronine uptake test as an example of an iodinated analyte. The method found to decolorize both hemolyzed and jaundiced samples is as follows:

1. Place the end-product of the radioimmunoassay (samples and standards) in a glass counting vial. Add 1.0 molar tetramethylammonium hydroxide and 30% hydrogen peroxide in amount indicated in Table 5.
2. Cap and vortex-mix thoroughly until the mixture is foaming vigorously.
3. Remove cap and let mixture react for 0.5 h, vortex-mix again, and visually check for complete decolorization. If not completely colorless, let react for another 0.5 h. Additional vortex-mixing may speed reaction.
4. Add HCl (3 mol/liter) and ascorbic acid (15 g/dl) in the amounts indicated in Table 5. Mix by swirling.
5. Add 15 ml of Beckman Ready-Solv VI to each vial. Cap and mix thoroughly by shaking or vortex-mixing.
6. Count on a liquid-scintillation counter and process the data by the usual procedure.

**Discussion of procedure.** Different volumes of reagents are required by different assays, depending on protein content, buffer volume, pH, etc. The proportions of ascorbic acid and hydrogen peroxide are the same, however.

Hydrogen peroxide will readily decolorize hemoglobin but a high pH is required before it will decolorize bilirubin. Before counting, the pH must be adjusted back to neutral or acidic to avoid chemiluminescence, because alkalinity promotes chemiluminescence (11). Either NaOH or KOH can be used with hydrogen peroxide to decolorize bilirubin, but the resulting salts upon neutralization cause problems in incorporating the sample into the emulsion liquid-scintillation cocktail. We used tetramethylammonium hydroxide as the base because the resulting salts are more soluble in the cocktail and its availability in a pure form. After decolorization with hydrogen peroxide is complete, HCl (3 mol/liter) is used to neutralize the base. The ascorbic acid in the formulation is added to scavenge the excess hydrogen peroxide, which may cause chemiluminescence owing to the liberated oxygen (12).

A number of types of cocktail were investigated; Ready-Solv VI gave the best results. This pre-mixed cocktail is also recommended because of its convenience. Although the suspension of the decolorized solution appears cloudy in the Ready-Solv VI, the counting efficiency was considerably higher than with other cocktails. Cocktails containing greater amounts of solubilizer gave clear solutions but lower counting efficiencies because of dilution and impurity quenching by the solubilizer. Substituting an additional quantity of the ascorbic acid solution for the HCl may give clearer solutions but may not totally suppress chemiluminescence. Ready-Solv VI and the decolorization procedure given here give cloudy solutions but exhibit less quench, as evidenced by higher ESCR's and higher counting efficiencies than other commercial preparations tested or procedures reported in the literature. An External Standard Channels Ratio number should be used to assess quenching in a sample, rather than its appearance.

**Recovery Experiments after Decolorization**

According to the protocol in Table 5, both hemolyzed and jaundiced samples were decolorized and recovery experiments for triiodothyronine uptake and digoxin assays were run.

Hemolyzed samples of known digoxin content were made up by adding Hycel Hemoglobin Control (lysed human erythrocytes) to A. R. Smith Digox-El control diluted fourfold with glycoside-free serum. The jaundiced samples were made up by diluting the control with Hyland Abnormal serum, which contained a supranormal bilirubin content.

The hemolyzed uptake samples were made up by adding Hycel Hemoglobin Control to Hyland Normal Serum Control. The jaundiced samples were obtained by different reconstitution volume of the Myland Abnormal Control Serum to give different elevated bilirubin concentrations. Predicted uptake

**Table 5. Decolorization Protocols for Hemolyzed and Jaundiced Samples Used in Digoxin and T-3 Uptake Assays**

<table>
<thead>
<tr>
<th>Tetramethylammonium hydroxide, mol/liter</th>
<th>For digoxin assay, ml</th>
<th>For T-3 uptake assay, ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0</td>
<td>0.3</td>
<td>0.5</td>
</tr>
<tr>
<td>H₂O₂, 30%</td>
<td>0.3</td>
<td>0.5</td>
</tr>
<tr>
<td>Reaction time</td>
<td>30 min</td>
<td>1 h</td>
</tr>
<tr>
<td>HCl, 3 mol/liter</td>
<td>0.3</td>
<td>0.3</td>
</tr>
<tr>
<td>Ascorbic acid, 15 g/dl</td>
<td>0.3</td>
<td>0.5</td>
</tr>
</tbody>
</table>

1192 CLINICAL CHEMISTRY, Vol. 20, No. 9, 1974
Table 6. Recovery of [14C] Digoxin Values after Decolorization of Hemolyzed and Jaundiced Samples

<table>
<thead>
<tr>
<th>Quenching agent</th>
<th>Undecolorized</th>
<th>After decolorn.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (no quench)</td>
<td>1.35</td>
<td>1.15</td>
</tr>
<tr>
<td>200 mg Hb/dl</td>
<td>3.70</td>
<td>1.30</td>
</tr>
<tr>
<td>800 mg Hb/dl</td>
<td>8.00</td>
<td>1.30</td>
</tr>
<tr>
<td>11.2 mg bilirubin/dl</td>
<td>3.75</td>
<td>1.20</td>
</tr>
</tbody>
</table>

* Sample: fourfold dilution of A. R. Smith, Digox-EI (1.05-1.65 ng/ml).

Table 7. T-3 Uptake Values Predicted and Found after Decolorization of Hemolyzed and Jaundiced Samples

<table>
<thead>
<tr>
<th>Sample</th>
<th>Quenching agent</th>
<th>T-3 Index, found</th>
<th>T-3 Index predicted*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control no. 1</td>
<td>400 mg Hb/dl</td>
<td>.86</td>
<td>.85 ± .02</td>
</tr>
<tr>
<td>Normal (Hyland)</td>
<td>800 mg Hb/dl</td>
<td>.85</td>
<td>.85 ± .02</td>
</tr>
<tr>
<td>No. 2 Abnormal</td>
<td>7.6 mg bilirubin/dl</td>
<td>.80</td>
<td>.79 ± .02</td>
</tr>
<tr>
<td>No. 3 Abnormal</td>
<td>14.8 mg bilirubin/dl</td>
<td>.87</td>
<td>.87 ± .01</td>
</tr>
</tbody>
</table>

* From duplicate samples counted on Biogamma.

values were determined by counting samples in a solid crystal gamma counter, which is not affected by quenching.

Results and discussion. As seen from Table 6, hemoglobin and bilirubin in serum cause erroneous digoxin results. Decolorization eliminates quenching and results in expected recoveries.

When jaundiced and hemolyzed samples used in the uptake tests were decolorized, expected results were obtained (Table 7).

Conclusion

A procedure was found that completely decolorizes both hemoglobin and bilirubin without causing significant quenching or chemiluminescence. Efficiency and recoveries are good with this procedure as used with 3H and 125I radioimmunoassays and radioassays, respectively.

The decolorizing procedure described above has the advantages of simplicity and speed. The reagents are available ready to use. All standards and samples are treated exactly alike, and no additional calculations are required. The disadvantage is that 15 ml of scintillation cocktail must be used for counting the assay to enable incorporation of aqueous solutions.

The automatic External Standard Channels Ratio method, on the other hand, requires no special reagents, no additional bench time for adding reagents, is used to correct only the significantly quenched samples that it automatically flags, and can be applied to samples counted by the Bio-Vial method with only 4 ml of scintillation cocktail. However, the External Standard Channels Ratio Method requires daily quench controls and calculation of the normalizing ratios. These extra calculations either require more technician time and chance for error or the use of computers. However, once familiarity with External Standard Channels Ratio is established, it is very practical, especially if calculations are incorporated as part of a computer program.

The decolorizing procedure may be preferred by laboratories that assay smaller numbers of samples.

Acknowledgments are due to Drs. Wm. F. Ulrich, Donald Horrocks, and Robert J. Obremski for valuable assistance.

References

5. Helman, E. Z., and Spiehler, V., Liquid scintillation counting for radioimmunoassays, Part II. Correlation of color quenching by instrumental methods. Beckman Clinical Brief 28 (see ref. 2).