The Use of Indigo Carmine for Dye Dilution Curves

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With the assistance of Thomas G. Arnold, B.S. and Lloyd H. Ramsey, M.D.

Investigation of the properties of indigo carmine reveals that it may be more suitable than the dye, T-1824, for circulatory studies that are now commonly carried out using the dye dilution principle of Stewart and Hamilton. Comparison is made of simultaneous dilution curves of indigo carmine and radioactive iodinated serum albumin. Apparently, indigo carmine is bound to protein firmly enough to remain in the plasma in one passage through the heart and lungs, but not too firmly to be extracted rapidly from the plasma by excretory systems in the kidney and liver. This allows repeated injections without accumulation.

Dye dilution methods for determining blood flow are used in a wide variety of circumstances. The slow excretion and undesirable discoloration caused by the most commonly used dye, T-1824, have limited the application of repeated injections in patients.

The ideal dye for repeated determinations of dilution curves should have the following characteristics: no toxicity; no loss of dye from the plasma from the point of injection to the point of observation; to be easily detectable in the blood by ordinary spectrophotometric methods; repeatability as often as desired, which, for a colored dye in patients, means that the dye should be eliminated rapidly from the plasma after it has passed through the segment of the circulatory system where the primary curve is formed.

The purpose of this paper is to report investigation of the dye, indigo carmine (Disodium 5,5'-indigotindisulfonate) with regard to the above characteristics.

Indigo carmine has been used, since its introduction in 1903 by Voelcher and Joseph, as a test of renal function and was the subject of investigation for many years. Although largely supplemented by phenolsulfonphthalein as a functional test, indigo carmine continues to be widely used at the present time in urologic examinations where its blue color allows ready identification of ureteral orifices at cystoscopy and confirmation of patency of the ureter.

Methods of Measurement

Solutions of the stock dye were made up in both water and serum to a concentration of 3.0 mg. per cent. The spectral absorption properties were determined, using a Coleman Jr. Model 6A Spectrophotometer, revealing a maximal absorption in the range of 600 to 610 mμ (fig. 1). The degree of absorption varied between water and serum solutions without a shift in the peak wavelength as has been reported for T-1824 and other dyes.

Standard serum solutions were then prepared in concentrations from 0 to 3 mg. per cent, at 0.5 mg. per cent intervals. The per cent transmission (%T) was read at a wavelength of 600 μ against a serum blank. Plotted against serum concentration,

two different pharmaceutical companies.* The material showed no visible precipitate when examined microscopically although visible precipitate has been reported in earlier preparations.1

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TABLE 1.—Comparison of Concentration of Indigo Carmine in Plasma Determination Colorimetrically after Addition of Known Amounts to Whole Blood

<table>
<thead>
<tr>
<th>Calculated Conc.</th>
<th>Conc. Read on Coleman</th>
</tr>
</thead>
<tbody>
<tr>
<td>.48 mgm.%</td>
<td>.49 mgm.%</td>
</tr>
<tr>
<td>.95 mgm.%</td>
<td>.95 mgm.%</td>
</tr>
<tr>
<td>1.40 mgm.%</td>
<td>1.39 mgm.%</td>
</tr>
<tr>
<td>5.36 mgm.%</td>
<td>5.70 mgm.%</td>
</tr>
<tr>
<td>2.19 mgm.%</td>
<td>2.24 mgm.%</td>
</tr>
</tbody>
</table>

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The rate of disappearance from the plasma was investigated by the intravenous injection of 5 cc. of 0.8 per cent solution into normal human subjects and determination of plasma concentration at three-minute intervals. In seven such instances, the logarithm of concentrations plotted against time showed a straight line relationship with half disappearance time averaging four and a half minutes (fig. 2). In one instance, urine collections were made and 18.9 per cent of the injected dye was recovered over a two-hour period.

Protein Binding

The protein binding of indigo carmine was investigated in two ways; by dialysis and by recovery from plasma when added to whole blood. Dialysis against saline was carried out at 37 C. for 24 hours by placing dog serum in Viscocele membrane bags, the dye being added to the serum in one instance and to the surrounding saline in the second. In both instances, 97 per cent of the dye was recovered in the serum.

Recovery from whole blood was determined by mixing known amounts of dye with canine whole blood. Hematocrits were determined and plasma concentrations were calculated from plasma volume, allowing 4 per cent for trapped plasma. The plasma was separated and concentrations read on the Coleman Spectrophotometer. The results are shown in table 1. Recovery of the dye from plasma varied from 94 to 101 per cent of the amount added to the blood.

Excretion

FIG. 1. A portion of the spectral-transmission curve of indigo carmine (read on a Coleman Jr. Spectrophotometer, model 6A.)

the %T showed a logarithmic relationship up to 4 mg. per cent concentration. Transmission varied for the same labeled concentration between some lots of stock dye so that a new standard curve was prepared for each new lot. The curves, however, were reproducible within lots. As expected from the spectral transmission curves, water and serum dilutions gave different curves. Determination of dye concentration in unknown samples was made by reading %T against blank serum and obtaining concentration from a standard curve.

FIG. 2. Curve of disappearance of indigo carmine from the plasma in normal human; ordinate is logarithmic scale.
Comparison of Indigo Carmine and RISA* Curves

The above studies indicate that indigo carmine can be measured in serum and suggest that the dye will not leave the plasma in its first circulation through the heart and lungs because it is bound to protein. To test this further, comparisons of simultaneous RISA and indigo carmine curves were made. Mongrel dogs weighing from 35 to 60 pounds were anesthetized with Xembutal. The jugular vein and femoral artery were exposed. A no. 10 cardiac catheter was introduced into the jugular vein and passed under fluoroscopic control to the pulmonary artery. A polyethylene cannula was introduced into the femoral artery and clamped until collections were made. Multiple successive samples were collected from the free flowing femoral cannula by allowing flow into 13 by 100 mm. test tubes, containing heparin, which were held in a lucite disc, rotating so as to bring the tubes under the cannula at a constant speed, approximately one tube per second.

Approximately 6 cc. of this mixture were injected rapidly into the catheter and sampling begun from the femoral artery. The catheter was then evacuated of dye by withdrawing with a syringe. The blood samples were centrifuged at low speed and plasma separated for analysis.

Each plasma sample of 2 cc. was pipetted into counting tubes, and activity was counted in a well type, sodium iodide, thallium activated scintillation counter, coupled to a Tracerlab utility scalar. In all determinations, 2 cc. samples were counted for 5 to 6 minutes and the average count per minute was calculated. Plasma was drawn before each injection for background counts. A 1:50 or 1:25 dilution was made of an aliquot of each injected solution and a 2 cc. sample of this was counted. From this, the counts per 2 cc. in the original solution injected were calculated. In order to compare the serum concentration of indigo carmine with the concentration of radioactive material, the ratio of the dye to quantities of RISA injected was used. Each serum value of RISA was multiplied by this ratio for quantitative comparison. The total amount of solution injected was not measured, as comparison between concentrations of the two substances was the only object of the evaluation. Two runs, approximately 30 minutes apart, were made on 5 dogs for 10 successive comparisons.

The concentration ratio of dye to RISA should be the same in the arterial blood as in the injected solution if both substances measure the same dilution process.

Results

A sample of comparative arterial concentrations is shown in figure 3. The mean difference ± standard error between 78 points was 0.027 mg. per cent ± 0.018 with a standard deviation of the differences of 0.158 mg. per cent. A significance test reveals this mean is not significantly different from a mean of 0 which would be expected if both methods measured identical phenomenon. Table 2 shows the comparative values for the total

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*RISA, radiiodinated serum albumin (131) was obtained from Abbott Laboratories.
TABLE 2.—Comparison of Areas under the Curves Calculated by Graphic Integration at Half Second Intervals

<table>
<thead>
<tr>
<th>Run</th>
<th>Area Indigo Carmine</th>
<th>Area RISA</th>
<th>Dye Area RISA Area</th>
<th>Dye RISA × 100</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>39.18</td>
<td>36.63</td>
<td>1.01</td>
<td>+1.0%</td>
</tr>
<tr>
<td>2</td>
<td>28.06</td>
<td>28.94</td>
<td>0.96</td>
<td>−3.2%</td>
</tr>
<tr>
<td>3</td>
<td>24.93</td>
<td>23.09</td>
<td>1.08</td>
<td>+8.0%</td>
</tr>
<tr>
<td>4</td>
<td>20.57</td>
<td>19.41</td>
<td>1.04</td>
<td>+3.5%</td>
</tr>
<tr>
<td>5</td>
<td>12.21</td>
<td>12.95</td>
<td>0.90</td>
<td>−3.1%</td>
</tr>
<tr>
<td>6</td>
<td>16.90</td>
<td>17.24</td>
<td>0.90</td>
<td>−2.0%</td>
</tr>
<tr>
<td>7</td>
<td>19.26</td>
<td>18.74</td>
<td>1.08</td>
<td>+2.6%</td>
</tr>
<tr>
<td>8</td>
<td>16.37</td>
<td>15.85</td>
<td>0.97</td>
<td>+3.0%</td>
</tr>
<tr>
<td>9</td>
<td>21.79</td>
<td>20.00</td>
<td>1.09</td>
<td>+8.9%</td>
</tr>
<tr>
<td>10</td>
<td>20.76</td>
<td>30.85</td>
<td>0.64</td>
<td>−3.6%</td>
</tr>
</tbody>
</table>

Mean difference is +0.99 per cent with a range of −3.6 per cent to +8.9 per cent.

Discussion

Comparisons of dilution curves of radioactive tagged albumin with T-1824 have shown agreement.\(^{11,12}\) If indigo carmine remains in the same vascular compartment as albumin, identical values of RISA and indigo carmine should be obtained, excluding recirculation. From the data, such appears to be the case.

Because of the original interest in dye dilution curves as a measure of the total plasma volume, little attention has been directed toward the more rapidly excreted dyes. However, to be useful in measurement of cardiac output, “central volume,” and estimation of intracardiac shunts, it is only necessary that the dye remain in the plasma during the first circulation. Indigo carmine appears to meet the requirements of being easily measurable in plasma and remaining in the vascular system in passage through the lungs and the heart. In addition, it has the desirable characteristic of being rapidly excreted. Apparently, it is not so firmly bound to protein that it cannot be extracted from the plasma by the kidney and liver.

Toxicity has not been extensively investigated in this laboratory but the dye has been widely used in urologic examinations for many years.\(^{5,7}\) A review of the literature since 1937 reveals only two reports on adverse reactions to indigo carmine. The only severe reaction reported was one of anaphylactic type resulting in severe respiratory and cardiac impairment.\(^{13}\) There was no mention in this report concerning analysis for impurities of the dye used for the injection. Hynson, Westcott and Dunning, Inc., one of the major suppliers of indigo carmine, reports that in the past 10 years there have been no reports to their laboratories of adverse reaction following intravenous injection of the dye. The 10 patients in our laboratory, receiving total doses of 24 to 106 mg. via intracardiac catheter for the detection of shunts, have had no adverse reactions. Indigo carmine solutions, unlike T-1824, cause no pain when subcutaneous extravasation occurs.

Although the major part of this investigation has been carried out in dogs, there is nothing to suggest the dye would behave differently in the human and the disappearance curves run in the human support this idea. However, comparison of concentration curves of RISA with indigo carmine in humans is in progress.

Summary

Indigo carmine can be quantitatively measured in serum by spectrophotometric methods. In vivo comparisons of simultaneous indigo carmine and radioactive iodinated serum albumin dilution curves in dogs reveal no significant difference during initial circulation. Indigo carmine is largely bound to serum protein and does not leave the vascular plasma compartment any more readily than albumin (RISA) during one circulation through the heart and lungs. Indigo carmine is removed from the plasma rapidly by the liver and kidneys and therefore is more useful for repeated determinations of dilution curves than T-1824.

Acknowledgment

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