Labeling with Indocyanine Green of Serum Protein from Normal Persons and Patients with Acute Viral Hepatitis

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Sera from normal persons and patients with acute viral hepatitis were mixed with indocyanine green (ICG), then fractionated on Sephadex G-200 columns. Alternate portions of the effluent were examined spectrophotometrically at 280 nm (for protein content) and at 800 nm (for ICG concentration). In most normal sera, three distinct ICG peaks were distinguished, the first and third corresponding exactly to the position of protein peaks, the second being most frequently shifted slightly toward heavier protein fractions. By graphic analysis, up to three additional masked peaks were usually detected in ICG curves. The individual fractions on ICG curves were numbered from I to VI, beginning with the fraction of greatest molecular weight. In the acute phase of viral hepatitis, the ICG curve was characteristically changed: fraction IV decreased significantly or even disappeared, and fraction I simultaneously increased. Fractions III and V also increased, but less. These changes in the ICG curves may result from interaction between serum proteins and liver cell proteins originating from damaged liver cells, an interaction leading to the formation of complexes of high molecular weight.

Additional Keyphases diagnosis and prognosis · Sephadex chromatography

Indocyanine Green (ICG) was introduced by Fox for the measurement of blood dilution curves (1). Shortly thereafter, Ketterer et al. (2) and Levey et al. (3) used ICG as a test of liver excretory function, following the work of Wheeler et al. (4). Later, Janecki and Seigert (5) stated that, during elimination of ICG from the circulation, they could observe several phases, which presumably were associated with different ICG affinities for different serum protein fractions.

To confirm this supposition, we mixed serum with ICG in vitro and then fractionated the proteins on a column containing Sephadex G-200.

The fractionation of serum, which has been mixed with an adequate amount of ICG solution, on a Sephadex G-200 column gives a curve for absorbance (at 800 nm) vs. volume leaving the column. This shows the distribution of the dye among the serum proteins.

During the last three years, we have investigated the qualitative as well as the quantitative patterns of such curves, obtained when sera from clinically normal persons and sera from patients with different pathological conditions, including many with acute viral hepatitis, are so examined. We briefly summarize our preliminary results here.

Materials and Methods

To a fresh serum sample, 0.1 volume of ICG in propanediol\(^1\) (250 mg/100 ml) was added. The mixture was applied to a column of Sephadex G-200\(^2\) (65 to 80-cm long, 10 or 18 cm\(^2\) in cross section) in the amount of 0.1–0.3 ml/cm\(^2\) of surface area, followed by 0.1M tris(hydroxyaminomethane or phosphate buffer, pH 8.0 (flow rate: 0.5 to 1 drop of buffer per cm\(^2\) of surface area per min). About 100 5-ml samples of protein containing effluent were usually collected (6,7). Absorbancies of alternate samples were measured with a Unicam SP 500 spectrophotometer at 280 and 800 nm, and plotted for successive samples. In this way, two elution curves were obtained, one for protein (280 nm) and one for ICG (800 nm).

\(^1\) ICG-preparat "Ujoviridin," Farbenfabrik Wolfen und-diluted with propanediol. ICG is available in the U.S. from Hynosin, Westcot, and Dunning, Inc., Baltimore, Md. 21201.

Table 1. Mean Content (and Range) of ICG in Individual Fractions of ICG Curves, for Healthy (Control) Group and Patients with Acute Viral Hepatitis

<table>
<thead>
<tr>
<th>Group</th>
<th>No. cases</th>
<th>Fractions, in % of total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>I</td>
</tr>
<tr>
<td>Healthy persons</td>
<td>14</td>
<td>14.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(2-27)</td>
</tr>
<tr>
<td>Acute viral hepatitis</td>
<td>24</td>
<td>34.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(12-58)</td>
</tr>
</tbody>
</table>

Thus far, 80 sera have been investigated, 14 from persons without any clinical and laboratory symptoms of liver damage or of dysproteinemia (control group), 32 from 24 patients with viral hepatitis, and 34 from other patients.

**Results**

With most normal sera, we obtained three distinct peaks (8) on the ICG curve, the first and third corresponding exactly to the peaks of the serum protein curve, and the second shifted slightly toward the protein fractions of greater molecular weight.

The basis for quantitative interpretation of results was the fact that the distribution of homogeneous protein fractions on a Sephadex column is very regularly symmetrical and corresponds to the normal (Gaussian) distribution curve. We have checked this fact several times after the gel filtration on a Sephadex G-200 column of bilirubin bound to albumin, or of hemoglobin. Therefore, it seemed reasonable to submit our ICG curves to graphic analysis.

So doing, we could detect additional masked peaks on ICG curves.

Usually we could distinguish six ICG-protein fractions with different gel-filtration speeds. The individual fractions on ICG curves were numbered I to VI, beginning with the fractions of largest molecular weight (Figures 1 and 2).

Table 1 demonstrates the mean content of ICG (in percentage of the total) in different peaks of ICG curves in the control group and in the group with acute viral hepatitis.

There are characteristic differences between the two ICG curves (Figures 1 and 2). In the acute phase of viral hepatitis, fraction IV decreases significantly or even disappears, with simultaneous increase of fraction I. Fractions III and V increase less. The more characteristic changes were observed in fraction IV. No case of acute viral hepatitis was found whose ICG content in fraction IV was as high as that in the control group, although in many cases the clinical and laboratory symptoms of the disease were only mild.

In three cases, the ICG content of fraction IV increased slowly during the recovery period, and reached its normal level during the fourth week after onset of the disease (8) (Figure 3).
In other pathological sera, including sera from patients with cirrhosis of the liver, the changes in the ICG curves were completely different from that observed in patients with viral hepatitis (Figure 4).

Discussion

Since 1959, Sephadex gel filtration (9) has been considered to be one of the most useful methods for protein investigation. Serum proteins were fractionated on Sephadex G-200 (Janecki, J., unpublished data) and their molecular weights estimated (10). Later, Sephadex gel filtration was used to investigate dye-binding capacity of serum proteins (11-15). Only once before was the distribution of ICG-labeled protein fractions in normal serum investigated by Sephadex G-200 fractionation (16).

Our present observations suggest that the observed changes during viral hepatitis may result from interaction between circulating serum proteins and liver cell proteins derived from damaged liver cells, thus leading to the formation of high molecular weight complexes. This could explain why fraction IV disappears and the heavier fractions, III and I, appear.

It is also possible that the binding of ICG may also involve two specific protein fractions of hepatic cytoplasm, designated by Levi et al. (17) as protein fractions Y and Z, which play an important role in the binding of bilirubin and of other dyes by liver cells.

Although all of these possibilities need experimental confirmation, our preliminary observations may have practical importance in the diagnosis and prognosis of viral hepatitis.
References


