from other drugs, and may therefore be preferable for pharmacokinetic studies, but a spectrophotometric procedure is suitable for round-the-clock management of acute asthmatic emergencies, as well as chronic care. The procedure described eliminates the most important and common source of interference, can be completed in 20–30 min, and is technically suited for emergency and routine use.

This work was supported in part by USPHS Research Grant No. 1 R01 MH19017 from the National Institute of Mental Health, NIH. I gratefully acknowledge the technical assistance of Miss Mullin Chiong.

References

Double-Antibody Radioimmunoassay of Serum Insulin: Effect of Use of Hormone-Depleted Human Serum

Cynthia K. Silbert and Clark T. Sawin

Human serum can be depleted of insulin and growth hormone by treatment with dextran-coated charcoal or by dialysis, or both. Inclusion of such hormone-depleted serum in the standard curve of a double-antibody radioimmunoassay for immunoreactive insulin more nearly mimics the conditions under which an unknown human serum sample is assayed. Compared to the usual standard curve in which serum albumin is the only protein, the addition of hormone-depleted serum can cause an increase (by an average 64% under the conditions we used) in the absolute value for apparent insulin in serum. The effect of hormone-depleted serum should be tested in the standard curves of double-antibody radioimmunoassays and included routinely in these standard curves if it changes the results. When used in conjunction with an eventual reference standard for human insulin, this modification of the insulin assay may make the measured values of human serum insulin from different laboratories more comparable.

In radioimmunoassay for immunoreactive insulin in serum, an unknown serum is compared to a standard curve prepared by use of increasing amounts of an insulin preparation that serves as a standard. The insulin standard is usually dissolved in a buffer solution containing bovine (1) or human (2) serum albumin as the only protein. The absence of human serum from the standard is thus an uncontrolled variable and might affect the absolute value of insulin determined in an unknown serum, particularly if it is assayed relatively undiluted. Because the absolute value for serum insulin is often used diagnostically (3), factors affecting its determination can be clinically important. Therefore, we examined the effect of insulin-depleted human serum on the standard curve of a double-antibody radioimmunoassay for immunoreactive insulin.

Methods

Preparation of hormone-depleted human serum. Human serum was treated with dextran-coated charcoal or dialyzed, or both.

Treatment with dextran-coated charcoal: (a) prepare a suspension of dextran-coated charcoal (4); (b) centrifuge at 10 000 x g for 10 min at 4 °C, beginning with a volume of charcoal suspension four times that of the serum to be
treated; (c) discard the supernate; (d) resuspend the pellet in the serum to be treated; and (e) centrifuge as before. On occasion, the last centrifugation may need to be repeated before the charcoal is completely removed.

Treatmen with dialysis: (a) place a measured volume of serum in boiled dialysis tubing; (b) dialyze against distilled water for three days at 4°C, changing the distilled water twice daily; (c) measure the volume of serum remaining after dialysis, as well as the sodium concentration and osmolality before and after dialysis; and (d) reconstitute the dialyzed serum to about the original osmolality and sodium concentration by use of appropriate amounts of Earle’s balanced salt solution (10X; Grand Island Biological Co., Grand Island, N.Y. 14072).

When serum was both treated with dextran-coated charcoal and dialyzed, dialysis and reconstitution were performed first. After treatment, we froze the serum for several weeks before we used it. When hormone-depleted serum was used in the radioimmunoassay for immunoreactive insulin, the original serum was obtained from healthy adults after an overnight fast. When hormone-depleted serum was tested for loss of hormones after treatment with dextran-coated charcoal and (or) dialysis, serum was also obtained postprandially, so that relatively large amounts of endogenous insulin would be present.

Assessment of hormone-depletion. $^{131}$I-labeled insulin (51 Ci/g) and $^{125}$I-labeled growth hormone (60 Ci/g; Abbott Laboratories, North Chicago, Ill. 60064) were added to serum before treatment with dextran-coated charcoal and (or) dialysis and the loss of radioactivity was measured after treatment. In addition, loss of endogenous insulin and growth hormone (somatotropin) was assessed by radioimmunoassays for insulin (7) and growth hormone (8) where-in the unknown serum is diluted at least 20-fold in the final incubation mixture and the assay does not depend on a second-antibody reaction.

Effect of hormone-depleted serum on the double-antibody radioimmunoassay of immunoreactive insulin. Standard curves were prepared by use of known amounts of insulin following the method of a commonly used double-antibody radioimmunoassay for immunoreactive insulin (7), which, like many others (8, 9) is a modification of the method of Morgan and Lazarow (1). Unknown sera are usually assayed relatively undiluted in these assays. In the assay used here (7), unknown sera were assayed at a final twofold dilution during incubation. A standard curve prepared by use of insulin standard diluted in a borate buffer containing bovine serum albumin (7) was compared to a standard curve in which some of the borate buffer/albumin solution was replaced by thawed hormone-depleted serum.

The amount of hormone-depleted serum used was equal to the amount of human serum used when assaying an unknown sample. As carrier protein to assist precipitation, we used normal guinea pig serum in the separation step in both standard curves. A single human insulin standard was used (generously donated by the Lilly Research Laboratories; lot No. 516-73-33).

Results

Table 1 shows that 90% or more of added radiolabeled insulin or growth hormone—or of endogenous insulin (two initial concentrations) or endogenous growth hormone—is removed by dextran-coated charcoal or dialysis, or both. Similar results were obtained with other samples.

Figure 1 shows the effect of hormone-depleted serum on the standard curve.1 Addition of depleted serum increased the amount of radiolabeled insulin in the precipitate at each concentration of unlabeled insulin standard, indicating that a given amount of unlabeled insulin displaced less radiolabeled insulin from its antibody. The resulting shift in the standard curve would increase the apparent insulin value of an unknown sample of serum by an average of 64% (Table 2).

Discussion

The values obtained in radioimmunoassays may differ from the actual values because of uncontrolled variables. Thus, values obtained with different methods or in different laboratories may not be comparable. An example of an uncontrolled variable is our previous finding that the absolute value of serum insulin may differ with different standard insulins of human origin (10). Therefore, in a given assay the standard curve and the unknown serum being assayed should be tested under conditions as similar as possible. When this is done in a commonly used radioimmunoassay for insulin, and hormone-depleted serum is included in the standard curve, our findings indicate that the absolute

1 Alternatively, the data on the ordinate of this figure could have been converted to log percent precipitated, so that the curves become second-order curves, with correlation coefficients $r$ of −.99 for depleted serum and −.98 for buffer albumin. The ordina data could also be converted to the reciprocal of the percent precipitated; the curves now become linear and the $r$ values are 0.99 for both curves.

---

Table 1. Removal from Human Serum of Endogenous and Added Radiolabeled Insulin and Growth Hormone (GH) by Dextran-Coated Charcoal and (or) Dialysis

<table>
<thead>
<tr>
<th></th>
<th>Endogenous insulin, mili-units/liter</th>
<th>$^{131}$I-labeled insulin, cpm/ml</th>
<th>Endogenous GH, ng/ml</th>
<th>$^{125}$I-labeled GH, cpm/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>No treatment</td>
<td>24</td>
<td>100</td>
<td>11 175</td>
<td>21</td>
</tr>
<tr>
<td>Dialysis</td>
<td>13</td>
<td>57</td>
<td>—</td>
<td>4</td>
</tr>
<tr>
<td>Dextran-coated charcoal</td>
<td>8</td>
<td>6</td>
<td>335</td>
<td>5</td>
</tr>
<tr>
<td>Both</td>
<td>1</td>
<td>7</td>
<td>—</td>
<td>1</td>
</tr>
</tbody>
</table>

---

Fig. 1. Comparison of standard curves prepared with and without use of hormone-depleted serum

Units on abscissa are microunits

---

CLINICAL CHEMISTRY, Vol. 21, No. 10, 1975
value of apparent serum insulin is higher than when the usual standard curve is used. Although this effect may not be found in all assays, it suggests that the effect of hormone-depleted serum should be tested and use of depleted serum omitted only if it can be shown to have no effect. If it has an effect, hormone-depleted serum should be routinely used in the standard curve of the assay.

The clinical use of an assay for serum insulin often requires that the absolute value be measured, because a diagnosis may depend on the serum insulin being more or less than a given concentration (3). Thus the assay should be performed in a strictly standardized manner in which the standard curve mimics the unknown serum as closely as possible. Hormone-depleted serum is easily prepared and helps allow this to be done. Moreover, the use of hormone-depleted serum and the eventual use of a standard reference preparation of human insulin may allow results from one laboratory to be compared more meaningfully to those from another laboratory and to published values. Such a comparison is not now possible unless the laboratories involved actually exchange samples, a cumbersome practice for widespread use and one which may reveal unexpected differences rather than similarities in insulin values (11). A valid comparison would still depend on a sufficiently detailed description of the assay, which is often omitted (3, 12, 13).

References