

Fig. 1. Cross reaction of 5α -dihydrotestosterone (upper curve) with testosterone-binding antibodies in a radioimmunoassay for testosterone (lower curve) in the standard curves shown, bound, labeled antigen is plotted against picograms of steroid. Cross reaction is calculated by using the ratio of the steroid concentrations required to decrease the binding of label to 50% of the value at zero

with reference to any intermediate step in the assay.

A disadvantage of the "sequential saturation" method that is not discussed by Zettner and Duly is the disastrous effect it has on the specificity of the assay. The binding of antigen in the first incubation step, where the antibody is not saturated, is on a "first-come, first-serve" basis. Where labeled antigen has no chance to compete with the interfering substances, the latter will be estimated to the same extent as the authentic antigen. Where competition can take place, the interfering substances are estimated only to the extent that their affinities for the antibody approach that of the authentic antigen.

Experimental results obtained in this laboratory are shown in Figures 1 and 2. Both the increased gradient and the decreased specificity of the "sequential saturation" method (designated "non-competitive" in Figure 2) are evident. Cross-reaction is calculated by the method of Abraham (4).

Attainment of adequate specificity is one of the most important and difficult aspects of immunoassay. For this reason, the usefulness of "sequential saturation" methods in immunoassay is very restricted.

References

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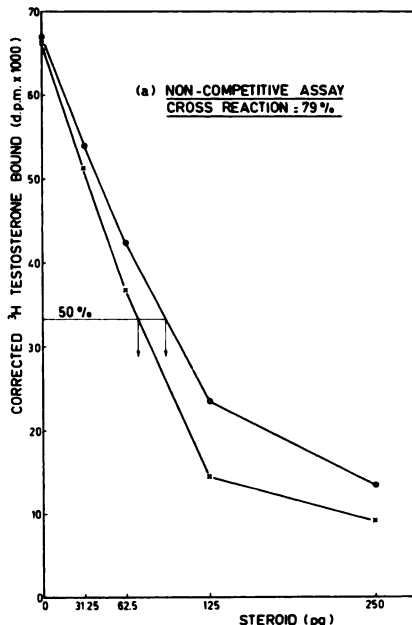


Fig. 2. A similar experiment to that represented in Figure 1 except that the nonlabeled steroid was incubated for 1 h with the antibody, and labeled steroid was added 5 min before separation of bound and free, i.e., a "sequential saturation" method was used

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Dr. Zettner responds:

To the Editor:

The concluding sections of our paper (ref. 1 above) dealt with the advantages and disadvantages of the sequential saturation method. There, we pointed out that the gains in sensitivity by this method are realized best with systems in which the concentration of reactants in terms of $1/K$ are high, a situation where greater sensitivity would be of little interest to the

analyst. The reproducibility achieved by the sequential saturation method is less than that of the equilibrium method only when the reactants' concentrations are low in terms of $1/K$. When they are high, reproducibility is experimentally indistinguishable from that of the equilibrium method. The more important advantages of sequential saturation method lie in areas other than sensitivity (see paragraphs II-V, pp 13-14, ref. 1).

The effects of cross-reacting ligands should be added to the list of disadvantages, while keeping in mind that this problem is not unique to the sequential saturation methods. The same effects, only less pronounced, are seen with equilibrium methods.

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Hepatitis-Contaminated Control Sera

To the Editor:

I wish to bring to your attention that quality control sera currently being marketed by several manufacturers are positive for hepatitis associated antigen (HAA). These materials should be considered biologically hazardous and not be used.

Certain manufacturers of these materials have taken a laissez faire attitude toward this situation by disclaiming responsibility, via package inserts, for injury caused by the use of these sera.

I remind every clinical chemist and laboratory director that they are responsible under OSHA regulations for maintaining a safe and healthful place of employment.

I urge you all to perform HAA tests, by third-generation techniques, on each lot of quality-control sera on hand in your laboratory. Return all of the positive ones to the manufacturer with requests for replacement. Report all positive lot numbers to your State's Department of Health.

Alan L. Portnoy
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Another View on Lipoprotein Electrophoresis

To the Editor:

In the March issue of *Clinical Chemistry*, it was suggested that lipoprotein electrophoresis be discontinued as a