Are We Accurately Quantitating Immune Complexes by Radioimmunoassay?

To the Editor:

In the last two decades the role of complexes of antigen and antibody (immune complexes, IC) in the pathogenesis of many diseases has become more and more evident, and numerous tests have been devised for detecting and quantitating circulating IC. Although there appears to be disagreement among results by various methods (1), IC are often assayed in clinical laboratories. Recently it was suggested that plasmapheresis can be effective for treating patients with systemic lupus erythematous (SLE), presumably by removing IC (2). Here the accuracy of tests for IC quantitation is crucial. In assays for quantitating IC, heat-aggregated human IgG with added complement is used as a standard, and the values obtained with patient's sera are expressed as IgG equivalent. In radioimmunoassays (RIA) for measuring IC, serial dilutions of aggregated IgG are used to construct standard curves, from which the concentrations of IC in sera can be determined. A widely used assay is the Raji cell RIA (3), in which lymphoblastoid cells are used to bind IC via their complement receptors. Radiolabeled anti-IgG (or protein A) is subsequently reacted with the Raji cells.

A criterion of the accuracy of an RIA is the parallelism between the binding curves obtained with serial dilutions of samples and with serial dilutions of the standard. Lack of parallelism of the concentration-reactivity curves leads to inaccurate results when the same sample is assayed at various dilutions. Patients with SLE often have high amounts of circulating IC, and their sera require dilution so that the amount of IC can be read from the standard curve.

We investigated whether the binding of IC from patients with SLE to the Raji cells is similar to that of aggregated human IgG to which complement (fresh human serum) was added. We diluted, with phosphate buffer containing bovine serum albumin, the sera from three patients who had high amounts of circulating IC in an initial screening test and assayed each sample for IC with the Raji cell RIA. The curves obtained with patients' sera were not parallel with the standard curve (Figure 1), as evidenced by the different results obtained with several dilutions of the same serum sample, e.g., 2000 µg of IgG equivalent at 1/4 dilution, 7520 at 1/8, and 10560 at 1/16 dilution of the same sample. Thus, the binding of complement-containing IC to the Raji cells appeared different from the binding of aggregated IgG to which complement was added. Perhaps there are different kinds of IC in various patients with SLE, which do not react identically with the Raji cells as does the aggregated IgG. The size of IC can vary with time, even in the same patients, and this puts in question the possibility of monitoring IC in patients who are undergoing various therapeutic measures such as hemodialysis (4) or plasmapheresis—i.e., accurate quantitation of IC is not possible. The heat treatment of IgG is known to produce various sizes of aggregates, but separating aggregates by size through gel filtration or ultracentrifugation gave similar findings.

Although RIAs for IC quantitation (e.g., Raji cell or Clq solid-phase assays) are useful tests for detecting small amounts of certain types of circulating IC, it should be remembered that, the value of these assays for quantitating IC with reference to aggregated human IgG is quite limited.

References
1. Lambert, P. M., et al., A WHO collabora-

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LD-1/LD Ratio as a Diagnostic Determinant for Myocardial Infarction

To the Editor:

We are currently conducting a study of the merits of many of the chemical tests used to assist in the diagnosis of myocardial infarction. We, therefore, read with great interest the paper by Bruns et al. (Clin. Chem. 27: 1821, 1981) in which they propose the use of the ratio of LD-1 to total LD (lactate dehydrogenase, EC 1.1.1.27) as a diagnostic determinant for myocardial infarction. We too have collected data from many patients in the Coronary Care Unit (CCU) whose diseases have been diagnosed by standard clinical criteria, so it seemed valuable to apply the LD-1/LD ratios to our data.

In our study, patients' sera was collected at first presentation, at 0800 hours the subsequent morning, and thereafter for two days. The data found to be of most value were those from the first two samples. In the cases we...
present here, the samples were at least 7 h apart but not more than 24 h apart, most differences being 12-17 h. LD-1 was determined by the Roche procedure, and LD was determined by using the pyruvate → lactate assay of Biomedix, at 30 °C. Calculations were based on using the highest value of LD-1 from each patient to discriminate between infarct and non-infarct.

In a population of 47 patients, 19 were considered not to have had infarcts (MI−) and 28 to have had infarcts (MI+). Selecting the best cutoff point for the ratio as 27.5%, there were six false positives and six false negatives (Figure 1).

This may be calculated to yield a predictability of 68.4% for no infarct or 78.5% for infarct. Although we concur that the proper use of LD-1 may facilitate the early diagnosis of a myocardial infarct, we have not found the LD-1/LD ratio to be nearly as predictive of myocardial infarct as have our colleagues. It is unfortunate, in both studies, that our samples are relatively small and biased, in that the patients were already selected for the CCU. A test of true predictibility ought to be done on a totally random assortment of patients from whom, unfortunately, it would be much more difficult to collect data.

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Naproxen Interferes Positively with 5-Hydroxyindoleacetic Acid Assay

To the Editor:

Over the last 18 months we have found increased urinary excretion of 5-hydroxyindoleacetic acid (5HIAA) in four patients who were taking an anti-rheumatoid drug, naproxen. We use the Goldenberg method (1) for 5HIAA, and the values found in these patients were unequivocally high, ranging from three- to sevenfold the upper reference value. When naproxen was withdrawn, excretion of 5HIAA returned to normal within three days. To confirm interference with the 5HIAA assay, two of us took naproxen; values for apparent 5HIAA in urine increased by four- to fivefold within two days.

Investigation of the urine by chromatography indicated that the increase in apparent 5HIAA was due to interference by a metabolite of naproxen in the nitrosanaphthol color reaction for 5HIAA, rather than an effect of naproxen on serotonin metabolism. We believe that all methods in which this color reaction is used will be so affected by the naproxen metabolite, including the widely used Goldenberg modification (conversion of the violet chromophore to blue by mercaptoethanol), a modification that has been regarded as highly specific for 5HIAA.

The only reference to this drug effect on 5HIAA excretion appears to be in the Proceedings of the 1976 Scandinavian Rheumatology Congress (2). We believe that this effect deserves to be more widely known in order to prevent extensive investigations for carcinoid syndrome, as happened in the first of our patients.

References


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Lack of Influence of Some Antibiotics on Various Thyroid Hormone Assays

To the Editor:

Because sera submitted for laboratory tests may contain antibiotic agents, we have examined the effect of some of them on assays for thyroid hormones.

We obtained 20 mL of venous blood from each of eight healthy white male volunteers and prepared 1.9 mL serum aliquots from each sample. These men were 25 to 30 years old, and had no history of prior thyroid disease or ingestion of medication during the preceding four weeks.

Laboratory-grade antibiotic powders (USP) were dissolved in appropriate buffers (Table 1) and stored at −70 °C. Control tubes containing only buffer were treated in an identical manner.

We added 0.1 mL of antibiotic solution or buffer to each serum aliquot, and kept the mixtures at room temperature for 1 h. The final antibiotic concentrations are recorded in Table 1. Antibiotics were allocated such that each agent was added to serum from four subjects and serum from each subject was assessed in the presence of four different antibiotic agents and corresponding controls.

All solutions were encoded with random numbers before submission for assay.

The following assays were performed during a single day by one technician, according to methodology supplied by the manufacturers: thyroxin (T₄) radioimmunoassay (Nuclear Medical Laboratories, Dallas, TX 75247); thyrotropin (TSH) radioimmunoassay (Beckman Diagnostics, Fullerton, CA 92634); triiodothyronine (T₃) radioimmunoassay (Nuclear Medical Laboratories); and T₃ resin uptake (Nuclear Medical Laboratories).

The result of each serum assay was within 5% of that for its control. Furthermore, all hormone concentrations were within normal limits for our laboratory, despite possible dilutional or pH artifacts introduced by the addition of antibiotic solutions.

A recently published review (1) of drugs that may affect thyroid function tests mentions only one antibacterial agent, p-aminosalicylic acid, which may interfere with measurement of T₄, free T₄ index, and presumably TSH. Moulding and Fraser (2) have identified additional instances in which hypothroidism resulted from ingestion of ethionamide, with depression of T₄ by both column and radioimmunoassay.

Each of the above reports suggests that these two drugs directly (rather than artifactually) affect thyroid hormone assay. This may, in turn, reflect the action of either the native drug or its metabolites on thyroid physiology, hormonal protein binding, or hepatic metabolism (3). Thus, penicillin has been shown to decrease the concentration of protein-bound iodine and T₄ (Murphy-Pattee method) through competition for sites on thyroid-binding prealbumin (4).

To date, no antimicrobial agent has been reported to interfere with radioimmunoassay for T₃ or T₄, unless the thyroid gland itself was affected. The present investigation suggests that therapeutic concentrations of commonly used antibiotic agents do not interfere with such tests.

References

2. Moulding, R., and Fraser, R., Hypothy-