of the sacrifice of some resolution as a result of the reprocessing.

The complex folding of the giant cell outer membrane (Fig. 2) was a characteristic feature. The tangential section (Fig. 3) also shows these folded membranes.

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Letters to the Editor

Automated Antibody (anti-D) Quantitation Technique

We were interested to read the article by Gunson, Philips, and Stratton (1972) concerning inherent errors within the automated antibody (anti-D) quantitation technique using the Technicon AutoAnalyzer. However, we are unable to agree with the authors' statement that non-linearity of graphs, obtained by plotting reciprocal of dilution against optical density, is due to loss of antigen excess (antibody excess).

The following calculations are based on the conditions occurring during the reaction phase using the technique described by us (Judd and Jenkins, 1970), and show conclusively that one is always operating with a vast excess of antigen: Assuming, (1) the molecular weight of IgG anti-D is 150 000, (2) the number of D sites per red cell (R_1R_1) is 20 000, a 20% red cell suspension will contain 2×10^9 red cells/ml.

According to Avogadro's number, 1 gram molecule of any substance will contain 6×10^{23} molecules, hence 6×10^{23} molecules of anti-D will weight 150 000 grams. Therefore, $1 \mu\text{g}$ of anti-D will contain

$$\frac{6 \times 10^{23}}{1.5 \times 10^5 \times 10^6} \text{ molecules}$$

ie, 4×10^{12} antibody molecules.

A 20% red cell suspension will contain $2 \times 10^9 \times 20\,000$ D antigen sites per ml, ie, 4×10^{13} D sites per ml.

In our experience an anti-D of $1 \mu\text{g}$ per ml requires to be diluted 1:100 for automated assay and this dilution will, therefore, contain 4×10^{10} antibody molecules per ml.

As equal volumes of red cell suspension

and serum are used in the AutoAnalyzer 4×10^{10} anti-D molecules per ml will be distributed amongst 4×10^{13} D antigen sites, that is to say 1/1000th of the total number of D sites may be combined with antibody molecules assuming that all antibody is bound. In order to obtain antibody excess within the AutoAnalyzer one would require more than 4×10^{13} molecules/ml, ie, $10 \mu\text{g}$ of anti-D/ml to be run through the system undiluted. If one postulates two antigen-combining sites for each antibody molecule then this figure can be halved, but at no time does one use this technique with such vast amounts of antibody.

We find a satisfactory standard graph can be obtained when operating within the range of 1:1000-1:4000 for a $20 \mu\text{g}/\text{ml}$ standard, ie, between 0.02 and 0.005 $\mu\text{g}/\text{ml}$. Under these conditions any non-linearity of the graph cannot be due to antibody excess.

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Our calculations show that almost complete agglutination is achieved in this system with approximately 25 antibody molecules per erythrocyte; an antibody concentration of about 0.018 μg per ml. Further increase in antibody concentration

leads to a proportional increase in bound antibody but does not materially affect the degree of agglutination. We note the authors now quote a range for the standard graph of 0.005 to 0.02 μg per ml whereas our comments related to their previous graph of 0.0115 to 0.115 μg per ml (Judd and Jenkins, 1970).

A large antigen site excess is required to maintain sufficient erythrocytes so that the number of bound antibody molecules per erythrocyte remains small. 'Loss of antigen excess', in this context, arises when the number of bound antibody molecules per erythrocyte is such that the degree of agglutination is no longer directly proportional to anti-D concentration and does not imply excess of antibody molecules over antigen sites.

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