Quantitation of Hæmagglutination by Enumeration of Free Cells by an Electronic Counter

Quantitative hæmagglutination methods[1] based on the enumeration of free or unagglutinated cells have given valuable information on the reactions of the antigens and antibodies of several blood-group systems[2]. These methods have had limited application, however, because of the tedium associated with visual counting techniques. To eliminate this objectionable feature, several investigators[3] have used the model *A* Coulter electronic counter to measure hæmagglutination in terms of the numbers of total particles, that is, aggregates and free cells, present in an agglutinated sample. The degree of hæmagglutination is then generally expressed as the percentage reduction in the unagglutinated total cell count.

Our earlier attempts to utilize the model *A* counter to provide information suitable for use in the quantitative hæmagglutination method of Wilkie and Becker[1], however, had shown that the relationship of percentages of reduction in total count to the logarithm of the antiserum concentrations could not be transformed by probits, logits or angles to a linear regression over a sufficient range of agglutination to validate an estimation of a 50 per cent end-point or HD_{50}. Moreover, attempts to establish a relationship between instrument total-particle and visual free-cell counts met with little success. Differences in mode of agglutinate formation among and within blood-group systems alter the relationship. Since it is not feasible to establish a relationship for each combination of antiserum and cell type to be examined, the model *A* counter was abandoned and an investigation initiated on the use of the model *B* Coulter counter to enumerate single cells in a population of agglutinates. This can be accomplished with the model *B* counter since it has two thresholds, the upper of which can be adjusted to eliminate aggregates of two or more cells from the count, thereby enabling true counts of the numbers of unagglutinated cells to be made.

During the past 9 months, the model *B* counter has been subjected to an extensive evaluation for suitability in providing quantitative data on the hæmagglutination reactions of the ABO systems. Agglutination produced by numerous avid and non-avid antisera from group A, B and O individuals reacted with A_1, A_2, A_X, A_1B, A_2B and B cells was measured by instrument and visual free-cell counts. It was found that there is excellent agreement in free-cell counts over the entire range of agglutination. This is shown in Table 1 which gives the results obtained by the two counting methods from the titration of an anti-A serum exhibiting a pronounced prozone with A_1 cells. It is also seen from Fig. 1 that when free-cell data are expressed as percentages of agglutination and plotted against the concentration of the antiserum in the manner described by Wilkie and Becker[1], there is no significant difference in the slope or position of the assay curves obtained by instrumentation and hæmacytometry.

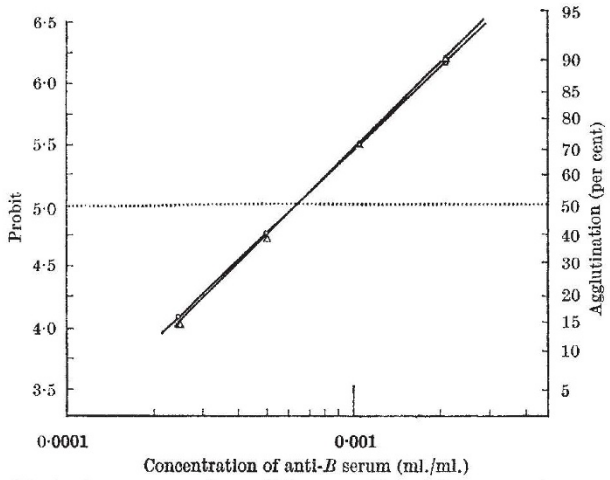

Fig. 1 Assay curves of an anti-B serum obtained by enumeration of free cells by hæmacytometry (△) and an electronic cell counter (○)

Table 1. Electronic Counter and Hæmacytometer Free-Cell Counts from the Titration of an Anti-A Serum* manifesting a Prozone with A_1 Cells

Dilution of antiserum (1:)	Free cells/mm³		Per cent agglutination†	
	Instrument	Visual	Instrument	Visual
2	8,840	8,750	32·5	33·2
4	7,034	7,100	46·3	45·8
8	3,675	3,408	71·9	74·0
16	1,705	1,768	87·0	86·5
32	604	640	95·4	95·1
64	246	295	98·1	97·9
128	192	190	98·5	98·5
256	504	450	96·1	96·6
512	1,718	1,720	86·9	86·8
1,024	3,979	3,966	69·6	69·7
2,048	8,040	7,450	38·6	43·1
4,096	10,300	10,500	21·3	19·8

* Serum from a group O individual immunized with human A_2 saliva substance.
† Total numbers of A_1 cells: 12,090/mm³.

A detailed description of the electronic method of counting single cells in an agglutinated sample is in preparation.

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Nitrite Reduction by Isolated Chloroplasts in Light

Losada, Whatley and Arnon[1] have shown that non-cyclic photo-phosphorylation in chloroplasts is the result of two independent light reactions: (*a*) a photo-oxidation of water, which is absent in photosynthetic bacteria; (*b*) a non-cyclic photo-phosphorylation of the bacterial type[2]. By inhibiting[1] or destroying[3] the system involved in the photo-oxidation of water, it has been possible to couple the light-dependent oxidation of certain suitable electron donors (ascorbate, cystein) with the photoreduction of such low-potential electron carriers as methyl or benzyl viologen[4]. These artificial co-factors can be used with the aid of bacterial hydrogenase either for the fixation or for the dark and light evolution of hydrogen gas[2,4,5]. Mortenson *et al.*[6] and Paneque and Arnon[3] have shown that ferredoxin is the natural electron transferring factor involved in these reactions. According to Tagawa and Arnon[7], the pyridine nucleotide reducing factors previously isolated and purified from either green plants[8,9] or photosynthetic bacteria[1] are ferredoxins.

Hageman *et al.*[10] have recently obtained an enzymatic system from leaves of *Cucurbita pepo* and *Zea mays* which catalyses the rapid and quantitative conversion of nitrite

Table 1. Photoreduction of Nitrite by Chloroplasts

Treatment	μMoles of nitrite reduced
Complete	3·7
Chloroplast extract omitted	1·3
Chloroplast extract boiled	0·7
Complete, dark	0·5

The reaction included, in a final volume of 3 ml.: washed broken chloroplasts heated at 55° C for 5 min containing 0·6 mg chlorophyll; chloroplast extract equivalent to 2 mg of chlorophyll; and the following in micromoles: *tris* buffer, *p*H 8·0, 150; sodium ascorbate, 20; 2,6-dichlorophenol indophenol, 0·2; sodium nitrite, 6. The reaction was carried out in Warburg vessels at 26° C for 25 min under argon. Illumination was from below by a fluorescent lamp of 100 W providing approximately 20,000 lux.