

LETTERS TO THE EDITORS

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An Apparatus for Counting Small Particles in Random Distribution, with Special Reference to Red Blood-Cells

THE advances which have been made in the measurement of hæmoglobin concentration have been largely offset from the clinical point of view by the continued inaccuracy of the red cell count. Hæmoglobin concentration can be estimated by quite simple apparatus with results that have a coefficient of variation of less than 3 per cent¹, whereas the corresponding figure for red-cell counting is about 9 per cent².

The error of red cell counting is made up of three components: (1) the diluting error, including the error due to wrongly dimensioned apparatus; (2) the unavoidable error due to the Poisson distribution of the cells in the counting chamber, which is a function of the square root of the number of cells counted; and (3) the error due to the human element.

An automatic counting apparatus could reduce the error due to (2) by counting a much larger number of cells than can conveniently be counted by eye and by eliminating the error due to (3). A counter for this purpose has been described by Lagercrantz³. It depends essentially upon a scanning system which is so arranged that the median particle diameter must be known to evaluate the result. This is an almost insuperable bar to its clinical application, since large and unknown variations in median cell-diameter occur naturally.

In an apparatus constructed in this laboratory, this difficulty has been eliminated. Let a plate on which is distributed at random a number of particles varying in size be scanned by an element of width greater than the diameter of the largest particle. Let every particle wholly or partially within the line produce a signal which can be counted. There will be some particles wholly within the line scanned and others overlapping into it from either side. If the line is very long, the number of such cells overlapping each boundary will be equal.

The actual area scanned is therefore a function of the width of the element, plus an undetermined area which is a function of the median particle-diameter. The latter is difficult to measure accurately, and varies from one blood sample to another. In order to eliminate this unknown factor, let the plate be scanned again by an element of greater width than before. The number of particles overlapping each boundary of this wider line will be the same as the number of particles overlapping the previous narrower one. Any increase in the count must therefore represent the number of particles present in the known additional area swept out by the wider element, irrespective of particle diameter.

In practice, the detecting mechanism will not be of infinite sensitivity. So long as the detector is sensitive to the smallest whole particle, and provided that the sensitivity stays constant for any two successive scans, the finite sensitivity will reduce the effective area scanned by the two elements by the same amount. This does not matter, as the final result is obtained from an area difference.

In the apparatus which has been operating in this laboratory, the sample is prepared from a suspension of red cells, made by diluting blood a known number of times, which is allowed to settle out of a known depth of suspension in a specially constructed counting chamber. A mechanical stage moves the chamber to and fro along its longitudinal axis, and along its lateral axis in such a way that a microscope objective fixed above the chamber scans a number of parallel lines. The length of the lines is governed by two blackened areas on the counting chamber, or by a circuit-breaker driven by the mechanical stage. The width of the scanning element is determined by two alternative slit-shaped stops, in the back focal plane of the objective. The illumination is provided by a high-power microscope lamp and a conventional condenser. The light transmitted by the stops is allowed to fall on to a photomultiplier, the signals from which are amplified and counted. Preliminary tests have shown that replicate red-cell counts made by the apparatus on the same specimen have a coefficient of variation of 2.1 per cent. The results of more extended practical trials at present in progress will appear elsewhere.

The same apparatus with or without small modifications can be used for counting any small particles which can be prepared in a distributed form and with sufficient contrast to their background.

Work on the apparatus described above was carried out at the instigation of Dr. R. G. Macfarlane, with financial assistance from the Medical Research Council and the Nuffield Foundation Hæmatological Research Fund. Considerable help from members of the staff of the Atomic Energy Research Establishment, Harwell, will be acknowledged in detail in a later publication.

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March 24.

¹ Macfarlane, R. G., King, E. J., Wooton, I. D. P., and Gilchrist, M., *Lancet*, i, 282 (1948).

² Biggs, R., and Macmillan, R. L., *J. Clin. Path.*, 1, 288 (1948).

³ Lagercrantz, C., *Nature*, 161, 25 (1948).

Total Cross-Section of Hydrogen and Carbon for 153-MeV. Neutrons

WE have used the 110-in. Harwell cyclotron¹ as a source of high-energy neutrons for total cross-section measurements in good geometry. An internal beryllium target 8.9 mm. thick was bombarded by about 1 μ amp. of 171-MeV. protons, and neutrons emerging near the forward direction were collimated by a series of 5-cm. diameter holes through three concrete screening walls. A polythene disk 19 mm. thick and 6.3 cm. in diameter was set up in the beam at a distance of 16 metres from the target. Protons ejected from the disk at an angle of 10° to the forward direction were detected by a triple-coincidence proportional counter telescope. Carbon absorbers placed between the counters in the telescope ensured that only protons with an energy greater than 141 MeV. were counted. This placed a lower limit of 145 MeV. on the energy of the neutrons detected, while an upper limit of 169 MeV. was set by the energy of the primary protons in the cyclotron. In a subsidiary experiment, the high-energy part of the