FIG. 2.

sufficiently numerous, they can be seen to form a white layer either just above or just below the red cells. With care, this layer can be pipetted off separately. Fig. 2 shows a smear of an eosinophil suspension prepared by this technique.

Of five patients with marked eosinophilia, two showed eosinophil bands above the red cells, two (including the example illustrated) showed eosinophil bands below the red cells, and in one the eosinophils were distributed at the same level as the red cells. It should be noted that these red cells are the residue after a preliminary dextran sedimentation, and it is probably these that vary in specific gravity rather than the eosinophils.

REFERENCE

Spriggs, A. I., and Alexander, R. F. (1960). *Nature (Lond.)* **188**, 863.

Technical notes on performing leucocyte counts on the EEL blood cell counter

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LEUCOCYTE COUNT DILUENT

The acetic acid-cetrimide-formalin diluent of Taylor and Rickards (1960a) has been used with satisfactory results after slight modification (see below). The diluent needs to have a low particle or 'blank' count for good reproducibility in the leucocyte counts, and as all the constituents of this diluent are liquid, they can easily be obtained dust-free by suction from the top of the contents of stock bottles which have been carefully stored undisturbed. Dust-free distilled water is collected through polyvinyl tubing from a glass-lined still direct into a storage aspirator via glass tubing in a rubber bung. The air-vent glass tube in the bung is covered with an inverted test-tube to prevent entry of dust. Dilution with this water provides a diluent ready for use without filtration. The diluent is stored in 2-litre aspirators with similar dust-traps, and sedimentation in these lessens still further the particle count to the equivalent of about 20 to 40 leucocytes per c.mm. It was found best to add the cetrimide to the other constituents after most of the distilled water had been added, otherwise a turbidity sometimes developed.

TUBES FOR THE LEUCOCYTE SUSPENSIONS

Disposable plastic tubes¹ are used, made of polystyrene with polythene plugs, 25.4 mm. long $\times$ 10.7 mm. internal diameter, capacity 2 ml. They are clean enough for use without washing and cost about 1d. each. A Speedry Capac brushpen² with a fine round nib is used to write indelible identification numbers on them.

The diluent is dispensed into these tubes in 0.95 ml. volumes (for 0.05 ml. blood samples giving a dilution of 1 in 20) with a stainless steel hand-operated ampoule filling machine³. This machine is reliable, quick to use, easy and quick to rinse after use, delivers these volumes $\pm 0.5\%$, and does not increase the particle count of the diluent. (Ground-glass stopcocks were found to increase

¹Obtainable from Luckham Ltd., 591/3 Kingston Road, Raynes Park, London, S.W.20.

²Model No. 57 obtainable from Speedry Products Ltd., 83 Copers Cope Road, Beckenham, Kent.

³Model No. 3 obtainable from A. J. Manning Ltd., Stonefield Close, Ruislip, Middlesex.

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the particle count markedly.) The blank count of the diluent in the unwashed tubes is equivalent to about 50 to 200 leucocytes/c.mm.

MIXING OF BLOOD AND DILUENT

Adequate mixing of blood and diluent is important for complete solution of red-cell stroma, especially when the blood has a high red-cell count. Inadequate mixing leaves clumps of 'ghosts' in the suspension. Taylor and Rickards (1960a) described a method of reducing the sensitivity of the counter so that such particles were not counted, but increasing the acetic acid concentration from 0.5% to 1.0% v/v and standardization of the mixing procedure was found to ensure the absence of these clumps. As most blood count work is carried out on venous blood samples sent to the laboratory, and to ease the preparation of leucocyte suspensions from these, a mechanical mixer is used. A carrier for the plastic tubes was fitted on to an Elliotts pipette shaker,⁴ so that the tubes are rotated in an oval orbit around their vertical axis. Each tube is mixed on this shaker at its slowest speed for 15 seconds. No alteration in the count results from this mixing. Suspensions prepared in the wards from capillary blood are given at least 20 complete hand inversions as rapidly as possible. New recruits to the laboratory staff need training in this procedure with artificially prepared 'polycythaemic' blood until microscopic inspection of the suspensions reveals adequate mixing.

Immediately before counting, the suspensions are put in turn on to a Matburn sloping turn-table mixer⁵ so that each is mixed for two to four minutes. Mixing on this type of mixer for longer than five minutes was found liable to increase the cell count, but no experiments were carried out to determine the cause.

With the sensitivity of the counter set as recommended by the makers, these leucocyte suspensions give satisfactory plateaux, the same counts being obtained at discriminator settings of 4 to 10.

1.0 C.MM. MICROPUMP

Both the 0.2 c.mm. and the 1.0 c.mm. micropumps are set by the makers to deliver 5% more than the stated volume to compensate for lost counts due to coincident pulses (Crosland-Taylor, Stewart, and Haggis, 1958). By comparison with visual counts, this calibration was found to be correct for normal red-cell counts with the 0.2 c.mm. micropump, and also for very high leucocyte counts with the 1.0 c.mm. micropump when readings of about 3,000 to 5,000 were being obtained. But when readings of about 250 to 500 were being obtained in normal leucocyte counts, the 5% extra volume was found to be unnecessary on our machine, the machine counts averaging about 5% higher than visual counts. In order

to have normal leucocyte counts carried out more accurately than high leucocyte counts, the 1.0 c.mm. pump was adjusted to deliver the stated volume.

After this calibration, machine and visual counts on 16 normal leucocyte suspensions were compared, the machine count being derived from four readings as recommended by the makers, and the visual counts from the cells counted in 0.8 c.mm. of the suspension. The former differed from the latter within a range of $\pm 9\%$ with an average difference of $+1.4\%$. The range of difference appears to be a manifestation of the combined random distribution errors of the two methods. This error in the machine was checked by carrying out duplicate counts on 80 normal leucocyte suspensions and all counts were within $\pm 4\%$ of the means of the paired counts. Because of this low error and as we report leucocyte counts over 4,000/c.mm. to the nearest 1,000/c.mm., the routine leucocyte counts are obtained by multiplying the sum of two readings by 10.

CIRCULATING WATER

When the counter was used only for red-cell counts, 0.04% v/v ammonia was a satisfactory circulating water. But when leucocyte suspensions in the diluent of Taylor and Rickards were passed into this alkaline water, a precipitate of denatured haemoglobin pigments was formed. The diluent circulating water used now with daily changes is 0.1% v/v acetic acid with 0.0004% cetrimide (0.001% v/v Cetavlon I.C.I.) at pH about 3.4. As this is based on the Taylor and Rickards diluent it dissolves red-cell stroma excellently, but does not prevent a slow deposition of protein inside the machine.

CLEARING TOP AND BOTTOM JETS

As described by Taylor and Rickards (1960b), a stainless steel three-way low-pressure stopcock has been fitted into the plastic tubing leading from the top jet. This greatly eases back-flushing of the top jet with water, when blockage or partial obstruction occurs, by means of a syringe attached to a female tubing-mount in plastic tubing on the side-arm. For this procedure the circulating pump is switched off and the by-pass valve held open.

A second T-piece is fitted into the micro-tubing just above the existing T-piece between the two micropumps. Micro-tubing tightly plugged at the other end with a stainless steel trocar is fitted to the side-arm and filled with water. This enables the bottom jet to be flushed with water without disturbing the micropumps. Care is necessary not to introduce air bubbles.

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REFERENCES

⁴Obtainable from Elliotts Liverpool Ltd., Buckland Street, Aigburth, Liverpool, 17.

⁵Obtainable from Matburn Ltd., 20-24 Emerald Street, London, W.C.1.

Crosland-Taylor, P., Stewart, J. W., and Haggis, G. (1958). *Blood*, 13, 398.
Taylor, F., and Rickards, A. G. (1960a). *J. clin. Path.*, 13, 249.
—, — (1960b). Personal communication.