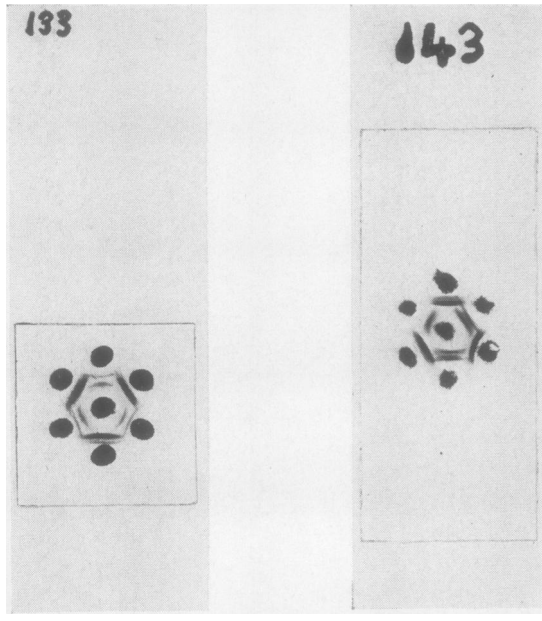

FIG. 3. *Examples of finished preparations, stained with 0·5% azocarmine.*

SUMMARY

A micro agar-gel precipitation technique is described in which the reagents are applied to the agar by means of a block of perspex through which holes have been drilled. The method allows a high degree of precision in placing the reagents on the agar with consequent good repeatability of results.

CORRECTIONS

We very much regret that Figs. 2, 4, and 7 of the paper 'Sarcoma of breast, with particular reference to its origin from fibroadenoma' by R. C. Curran and O. G. Dodge (*J. clin. Path.*, **15**, 1-16) have been printed upside down. In the legends accordingly please read 'right' for 'left' and vice versa. Also Figs. 12 and 13 refer to Case 37, not to Case 36 as printed.

Professor N. H. Martin asks that the last phrase of the last sentence of the first paragraph of the Discussion in his paper 'Serum sialic acid levels in health and disease' (*J. clin. Path.*, **15**, 71) 'whereas an increase in the α fraction would have little effect on this ratio', be deleted.

A method for obtaining concentrates of eosinophils from blood

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In a previous publication (Spriggs and Alexander, 1960) we described an albumin gradient method for separating the different white cells of blood. This was applied to the isolation of tumour cells but it was also noted that very pure suspensions of neutrophil polymorphonuclears could be obtained by the same method. It has now been found that the eosinophil leucocytes can be collected by the albumin method in a separate layer, and if the blood sample comes from a patient with eosinophilia it is sometimes possible to pipette these cells off in a high degree of purity.

The albumin gradient method need not be described again, but it should be noted that all equipment *must be siliconed*. The cells form a series of layers according to their specific gravity, as shown in Fig. 1. The platelets

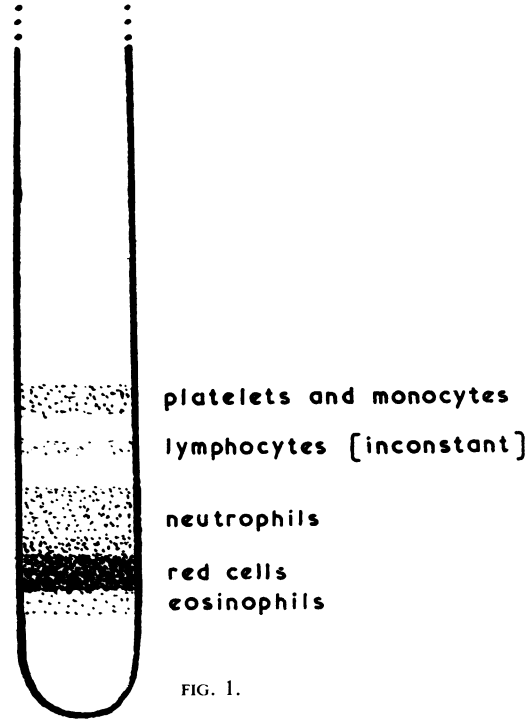

FIG. 1.

are on top, with most of the leucocytes below them, and any residual red cells not removed by the preliminary sedimentation are lower still. When eosinophils are

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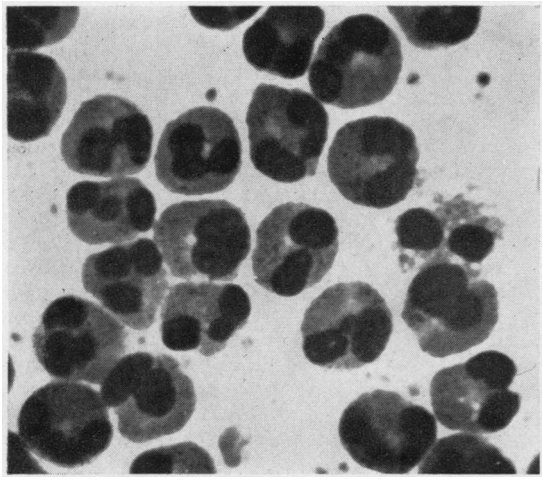

FIG. 2.

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 tubes[1] are used, made of polystyrene with polythene plugs, 25·4 mm. long × 10·7 mm. internal diameter, capacity 2 ml. They are clean enough for use without washing and cost about 1d. each. A Speedry Capac brushpen[2] 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 machine[3]. This machine is reliable, quick to use, easy and quick to rinse after use, delivers these volumes ±0·5%, and does not increase the particle count of the diluent. (Ground-glass stopcocks were found to increase

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

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

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

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