A method for obtaining concentrates of eosinophils from blood

R. F. ALEXANDER AND A. I. SPRIGGS From the Churchill Hospital, Oxford

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.

platelets and monocytes
lymphocytes [inconstant]
neutrophils
red cells
eosinophils

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

Received for publication 20 July 1961.

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.

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.

FIG. 3. Examples of finished preparations, stained with 0.5% azocarmine.
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
element 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

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

J. G. SELWYN From the Department of Pathology, West Middlesex Hospital, Isleworth

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 reproduc-

ibility 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 leuco-
cytes 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 \(\times\) 10·7 mm. internal
diameter, capacity 2 ml. They are clean enough for use
without washing and cost about 1d. each. A Speedry
Capuchin\(^2\) 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
\(\pm 0·5\%\), 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,

\(^2\) 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.

Received for publication 30 October 1961.
A method for obtaining concentrates of eosinophils from blood

R. F. Alexander and A. I. Spriggs

doi: 10.1136/jcp.15.2.188-c

Updated information and services can be found at:
http://jcp.bmj.com/content/15/2/188.3.citation

These include:

**Email alerting service**

Receive free email alerts when new articles cite this article. Sign up in the box at the top right corner of the online article.

**Notes**

To request permissions go to:
http://group.bmj.com/group/rights-licensing/permissions

To order reprints go to:
http://journals.bmj.com/cgi/reprintform

To subscribe to BMJ go to:
http://group.bmj.com/subscribe/