EVALUATION OF AN IMPROVED METHOD FOR DIRECT BASOPHIL COUNTS*

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SUMMARY

The improved method for direct basophil counts described by Cooper and Cruickshank was evaluated. Their findings, indicating a small variance and negligible chamber error, could be confirmed on a larger series of normal individuals.

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There is little doubt that one of the contributing factors to the scantiness of knowledge on basophil leucocyte function is the lack of a satisfactory direct counting method to enumerate these cells in capillary and venous blood.

The indirect method, making use of differential counts to calculate the 'absolute' number of basophils as a percentage of the total leucocyte counts, is satisfactory only when at least 2 000 cells are classified in the differential count.[1]

Since the first description[1] of a method for direct basophil counts in 1953, several modifications[2-4] of the method points to its inadequacies. Although all methods employ toluidine blue for metachromatic staining of basophils, our own experience and that of others, found the two main problems to be the diffuse staining due to water solubility of the polysaccharides in basophil granules and the clotting and aggregation of blood platelets, frequently associated with degranulation of basophils.[5]

These problems were overcome by Cooper and Cruickshank[5] by adding cetylpyridinium chloride and aluminium sulphate to the toluidine blue. The former lyses erythrocytes and complexes with mucopolysaccharides, rendering them relatively insoluble in water. The latter accentuates the metachromatic staining by acting as a mordant. The use of EDTA in saline as anticoagulant prevents aggregation and platelet clumping.

METHOD

To assess the improved method of Cooper and Cruickshank[5] we employed the same experimental set-up as used by them to assess errors associated with pipetting and counting. An attempt to reduce pipetting errors was made by using a screw-attachment attached to the pipettes for measuring out the diluents. This was compared with pipetting done by mouth through a rubber tube in a separate experiment on a single blood sample.

Blood was obtained from a fingerprick in 37 individuals. The first drop was wiped away and 0·02 ml of the second and subsequent drops were taken with 3 pipettes. This was added to 0·08 ml 0·1% EDTA previously delivered into Wassermann tubes. To the anticoagulant in the Wassermann tubes was added 0·1 ml of the cetylpyridinium chloride-toluidine blue-aluminium sulphate solution, and the tube gently shaken. Two Fuchs-Rosenthal counting chambers were filled from each of the three samples obtained from each individual with a Pasteur pipette and the stained basophils counted. For each individual we therefore had 6 separate counts.

STATISTICAL ANALYSIS

A three-factor analysis of variance was carried out on the counts. The counting chambers (C) and patients (P) are two factors occuring together throughout, while the three pipette samples (S) are nested within patients. The three pipette samples were considered as random samples from an infinite population of possible blood samples from each patient. The patients were considered as a random sample from an infinite population of possible patients. The counting chambers were two specific chambers from which conclusions were to be drawn.

If Y_{IJK} represents the number of basophils per cubic millimetre blood from the k-th sample of patient i, as counted in chamber j, then i=1 . . . 37, j=1,2 and k=1,2,3 in this set of data. A suitable model for analysis of variance would then be:[6]

$$Y_{IJK}=\mu+a_I{}^P+\alpha_J{}^C+a_{IK}{}^S+a_{IJ}{}^{PC}+a_{IJK}{}^{CS}+e_{IJK},$$

where the a's are main effects and interactions of the random factors while α^C is the only fixed effect. The e_{IJK} are the error components which are assumed as independent and normally distributed. The analysis of variance for this model is shown in Table I.

TABLE I. ANALYSIS OF VARIANCE

Source of variation	*Sum of squares*	*Degrees of freedom*	*Mean sum of squares*	*F-tests*
Between patients	81 481·56	36	2 263·38	34·7
Between counting chambers	100·81	1	100·81	1·77
Between samples (within patients)	4 825·50	74	65·21	
P×C interaction	2 045·13	36	56·81	1·09
C×S interaction (within patients)	3 871·44	74	52·32	

The residual variance is thus 52·32. The mean, $\bar{x}$, of any future patient, calculated from 6 observations in 2 counting chambers, would thus have a standard error of $(52{\cdot}32/6) = 2{\cdot}95$. A confidence interval for the true mean of a future patient would then be $\bar{x} \pm 2{\cdot}95\ t$, where t refers to student's t with 74 degrees of freedom.

The components of the total variance that can be attributed to samples and counting chambers are in the relation of 47·9:1. The mean counts for the two counting chambers were 33·4 and 34·7 and the mean for the total sample of patients was 34·1.

In the experiment where 10 counts were performed over 6 counting nets on dilutions made by the screw-attachment, and the same procedure carried out on dilutions made by mouth-pipetting, no significant advantage is apparent in either procedure. The means and standard deviations were $2{\cdot}7 \pm 1{\cdot}49$ and $2{\cdot}5 \pm 1{\cdot}45$ cells per counting net respectively.

DISCUSSION

This evaluation of Cooper and Cruickshank's improved method for direct counting of basophils confirms their findings.[5] The small variance found by them could be confirmed on this larger series of patients and makes the method very suitable to detect minor changes in basophil counts. There appears to be no method at present available to further reduce the major potential source of error, namely that inherent to pipette-dilutions. Like the original authors, we also believe that the improved staining technique is responsible for the reduction of total error.

As suggested by Cruickshank and Haye,[7] the tendency 'to translate basophil function in terms of mast cell behaviour during the whole of this century' can, with this improved method, now be changed to have 'a new look at basophils in their own right'.

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