A MECHANICAL AID IN MAKING BLOOD FILMS

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The apparatus which forms the subject of this paper was designed to eliminate some of the variable factors during the preparation of blood films by the slide method, and thus to reduce the errors of differential leucocyte counts. These errors can be grouped, according to their sources, into those arising during the preparation of the films due to a variety of technical factors, and into others inherent in every process of sampling, in which percentages of a total population are estimated from an analysis of small fractions. Efforts may justifiably be made to reduce the size of the first group of errors; that of the second is irreducible.

The distribution of leucocytes on blood films is irregular, there being a relative excess of cells along the edges and at the end, an excess to which the various cell classes contribute to a varying extent: the larger a leucocyte, the more likely is it to be carried to the edges or the end (Gyllenswärd, 1929). Since lymphocytes are the smallest leucocytes, there is thus a relative lymphocytosis in the centre, and a relative lymphopenia at the edges of the film. MacGregor, Richards, and Loh (1940) have shown that a "battlement" count, which takes in parts of the edges and the adjacent interior, largely obviates this source of error, and gives results closely approximating those of counts of all leucocytes in the film.

Many factors, other than differences in cell size, may influence the quality of blood films and contribute to irregularities in the distribution of leucocytes. Thus the size of the drop of blood used, the speed of spreading, the width and nature of the spreading edge, the angle at which it is held, and the pressure applied, may all vary from observer to observer, and from occasion to occasion.‡ This problem has been analysed by Mainland, Coady, and Joseph (1936), who conclude that while it is impossible to incriminate any single factors, their combination leads to irregular spreading: "The variation . . . can be attributed to minor differences in cell size, and, perhaps, local variations in the current of the blood as it flows over the slide, which are uncontrollable factors."

The most important result of the irregular leucocyte distribution is the fact that a large number of cells have to be counted in order to ensure reasonable accuracy.

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‡Fundamentally the thickness of films and distribution of cells depend on local electrostatic and surface tension or capillary forces in relation to the wettability of the glass surface. If it were possible to obtain a surface, perfectly wettable by blood, the cell distribution should be entirely regular.

Thus Mainland, Coady, and Joseph (1936) have shown that, if three counts of 100 cells are done on the same films, none of them is equivalent to any of the others, even if no count includes cells from the "tail" of the film. It is usually recommended (e.g., by Beacom, 1928, and Medlar, 1931) that 300 or 400 cells should be counted as a routine. This figure might be expected to be reduced if the distribution of cells could be made more regular, so as to approximate to that of a binomial distribution. The sole remaining error would then be that inherent in sampling, and the size of the latter could be easily calculated (Goldner and Mann, 1938).

The Apparatus

The apparatus* permits the making of blood films on glass slides, keeping constant the width and consistence of the spreading edge, its angle with and pressure on the slide. This is achieved by moving the slide on a carriage under the pre-set and fixed spreader. A diagrammatic view is shown in Fig. 1.

FIG. 1.—The apparatus described for making blood films.

* The original models of the apparatus were made in the Departments of Radiotherapeutics and Medicine, Cambridge University, by Mr. V. D. Martin and Mr. J. R. Flemans.

The spreader consists of a portion of a standard three-hole or slot razor blade and is held tightly in the metal blade holder. This blade holder is attached to a bar which runs through the slide support and has attached to its far end a knurled nut by means of which it can be rotated. Also attached to the blade is a stop which impinges on an adjustable stop on the side support. These stops fix the blade depth in relation to the slide. A weak spring is enclosed in the side support and attached to the knurled nut. It is adjustable for tension by rotation of the knurled nut, which in its turn is attached to the blade holder bar by a lock screw and provides the blade pressure on the slide.

The spreader is mounted on a side support on a troughed carriageway which has stops fitted at each end of the groove. Into this groove fits the slide carriage, which is recessed to hold the glass slide and has a knob at one end for easy traction.

The spreader holder is of necessity made of metal, but the carriage and carriageway can with advantage be made of "perspex," which provides a light, clean finished article.

The apparatus measures about $15 \times 7\frac{1}{2} \times 4\frac{1}{2}$ cm. and is easily portable.

Use of Apparatus.—A small drop of blood is placed at one end of a glass slide. This is best spread out in a thin line corresponding to the width of the blade. The slide is then placed in the slide carriage with the blood lying under the spreader holder. The carriage is drawn back until the stop is reached, thus causing the blood to form a thin film under the spreader edge. It is then moved with a steady slow movement in the opposite direction, and a thin film of blood is left on the slide. The thickness of this film can be varied by altering the amount of blood used and the speed with which the carriage is pushed. Provided the drop of blood used is small and the blade clean, the latter requires no cleaning after spreading, for no blood adheres to its edge. It should be renewed approximately once a month, when its edge becomes worn. A worn edge is shown by the presence of distortion and tearing of the cells along the edge of the film.

Films spread by this apparatus show absolutely straight edges, thus facilitating the performance of "battlement" counts. Their initial parts (about $1/3$) tend to be thicker than those of hand-spread films, while the distal parts have the usual thickness and should be used for counting. The "tail" is often shorter than in hand-made films. The apparatus is easy to use, and even an inexperienced person can make satisfactory films with it after a very few trials.

Results

Two separate investigations were made in order to assess the value of the apparatus: (1) An analysis of cell distribution; (2) an investigation of cell size.

Analysis of Cell Distribution.—Venous blood from five normal individuals was used. After thorough shaking, 10 films were prepared from each blood by the same observer; of these five were made by hand, and five by the machine. There were thus available a total of 25 machine-made and 25 hand-made films.

Five differential counts were performed on each slide, employing the "battlement" procedure. Each single count was of 100 cells, classified into neutrophils, lymphocytes, monocytes, eosinophils, and basophils. In order to minimize personal bias which is liable to arise when the observer knows some of the previous results, all counts were recorded by an assistant, the observer remaining ignorant of the results until the end of the test. All counts were performed by the same individual.

The mathematical analysis given below has been made on the percentage of granulocytes present. A more refined analysis, which would deal with a suitable function of the percentage, was thought unnecessary, in view of the clear-cut results obtained. The aim was to compare the degree of homogeneity of the cell distribution in the hand-made and machine-made films.

For the purpose of analysis, we may first compare the cell distribution on a given slide with a binomial one, by means of Pearson's χ^2 test. For each slide we have five counts, each distinguishing two classes: granulocytes and the rest; this will give a χ^2 with four degrees of freedom. We can combine these for the five slides of each of the five bloods to give a composite χ^2 with 100 degrees of freedom. The values obtained are:

$$\begin{array}{l} \text{Hand method } \chi^2_{100} = 221.7 \text{ } P < 0.1\% \\ \text{Machine method } \chi^2_{100} = 121.7 \text{ } P = 6.8\% \end{array}$$

The value for the hand method is highly significant, and hence the cell distribution in hand-made films shows a highly significant departure from a binomial one. The distribution in machine-made films, on the other hand, shows no significant departure from a binomial one, accepting the customary 5% level of significance.

If we now analyse the total counts of 500 cells for each slide, we can calculate for each of the five bloods a χ^2 with four degrees of freedom. Adding the quantities for the different bloods gives a χ^2 with 20 degrees of freedom. The results are:

$$\begin{array}{l} \text{Hand method } \chi^2_{20} = 23.6 \\ \text{Machine method } \chi^2_{20} = 20.3 \end{array}$$

Neither value is significant.

This analysis shows that as far as the proportion of granulocytes is concerned, there is a binomial distribution for both methods of spreading, if we consider large counts of the order of 500 taken from each slide. The data from different slides appear to be homogeneous, and we can calculate the standard errors in the usual way. On the other hand, if we look at several counts of 100 cells drawn from a single slide, then while there is probably still a binomial distribution in the machine-made films, this is definitely not so in films spread by the old method.

It is possible to examine the same data in a different way, by analysing the degree of irregularity in results due to variation between slides and within slides. This is done in the analysis of variance in Table I, which shows that with the machine

TABLE I

ANALYSIS OF VARIANCE OF PERCENTAGE OF GRANULOCYTES IN 125 COUNTS OF 100 CELLS IN HAND-MADE AND 125 IN MACHINE-MADE FILMS

Source of Variation	Old Method			New Method		
	Sum of Sq.	D. of F.	Mean Sq.	Sum of Sq.	D. of F.	Mean Sq.
Within slides ..	5026.40	100	50.26	2711.20	100	27.11
Between slides ..	509.68	20	25.48	463.04	20	23.15
Between bloods ..	2364.85	4	591.21	1720.05	4	430.01
Total ..	7900.93	124		4894.29	124	

method the degree of variation within slides does not differ significantly from that between different slides. But with the old method the variation within slides is twice as great as the variation between slides: the difference is significant, $P < 5\%$.

Thus if we employed single counts of 100 the variation would be approximately twice as great using the old method of spreading. It is pertinent to ask whether this disadvantage is overcome before reaching the count-size of 500, at which, as we have seen above, the distribution can be taken to be binomial. In order to answer this question, the first two counts on each slide were selected and added together, making a total count of 200. The variation of these counts about the blood means was then examined and the variance was found to be approximately 77 compared with the 54 expected from the new method. Thus for counts of 200 the old method has about 70% of the efficiency of the new method. Counts of 300, 400, and 500 showed no difference between the methods.

The advantage of machine-spread slides is therefore that we can assume a binomial distribution for leucocyte counts as low as 100, and thus regard such counts as representative of the overall distribution on the slide; further, the variation within single slides and between a series of films of the same blood is about the same, so that a differential count of 100 leucocytes is representative of the whole series and can be used for calculating the remaining statistical error and establishing confidence limits. With hand-made films, counts of 100 or 200 cells are respectively only 50% and 70% as accurate as counts of the same size on machine-made films; if 300 cells are counted, no difference is found between the two methods. The analysis of films made by hand confirms the findings of Mainland, Coady, and Joseph (1936), and also supplies new evidence in support of the frequently voiced opinion that at least 300 cells should be counted to obtain a reasonably accurate result in routine counting.

Investigation of Cell Size—The size of leucocytes is occasionally measured in blood films, and it is reasonable to assume that the values found depend partly on the amount of distortion of individual cells introduced by the process of spreading. The aim of this investigation was to compare the degree of distortion in hand-made and machine-made films.

Normal venous blood was obtained from the same individual on two different occasions. On the first, five films were made by machine and four by hand; on the second four films by machine and four by hand. The hand-made films were prepared by different skilled workers on the two occasions. There were thus available nine machine-made and eight hand-made films, the latter subdivided into two groups of four, made respectively by workers W_1 and W_2 .

On each film, the size of 100 consecutive neutrophils was determined by means of a micrometer eyepiece, the mean of two readings at right angles being expressed in arbitrary scale divisions. In all films cells were measured in the same order, by moving from one edge to the other across the middle of the films, until the full 100 cells had been included.

The data analysed are the mean and standard deviation for each slide. These are given in Table II.

We can employ Student's *t*-test to test the significance of the differences between the means of the various groups in which we are interested, using an estimate of

TABLE II
SIZES OF NEUTROPHILS COMPARED IN MACHINE- AND HAND-MADE FILMS

	Type	Slide	Mean	Standard Deviation
Machine-made	T ₁ *	M ₁	17.55	1.23
		M ₂	17.86	1.01
		M ₃	17.76	1.42
		M ₄	18.35	1.34
		M ₅	18.28	1.33
	Average T ₁	=	17.96	1.27
Hand-made	T ₂	M ₆	17.70	1.14
		M ₇	18.34	1.12
		M ₈	17.45	1.14
		M ₉	17.48	1.02
	Average T ₂	=	17.74	1.10
Machine-made	W ₁	H ₁	19.13	1.51
		H ₂	18.84	1.49
		H ₃	20.04	1.24
		H ₄	18.88	1.41
	Average W ₁	=	19.22	1.41
Hand-made	W ₂	H ₅	18.47	1.21
		H ₆	19.15	1.17
		H ₇	19.76	1.22
		H ₈	19.03	1.01
	Average W ₂	=	19.10	1.15

* Slides T₁ and W₁ made from one sample of blood, slides T₂ and W₂ from a later sample of the same blood.

variance based on the total of 13 degrees of freedom within the four main classes—i.e., four from T₁ and three from each of T₂, W₁, and W₂.

Among the means the only difference which is significant is that between the averages for hand-made and machine-made—i.e., $19.16 - 17.86 = 1.30$. The standard error of the difference under consideration is 0.224. This makes $t = 5.80$; with 13 degrees of freedom this is significant with $P < 0.1\%$.

Among the standard deviations, again only one difference is significant—that between the averages for the two operators—i.e., $1.41 - 1.15 = 0.26$. The corresponding standard error is 0.084, and thus $t = 3.10$; with 13 degrees of freedom this is significant with $P < 1\%$. The value of t for the difference between the averages for the two machine-made groups is 2.02, for which $P = 6.4\%$: the difference, though substantial, is not significant.

The analysis as a whole suggests that the mean size of 100 cells from a given slide is somewhat smaller when the slide is machine-made. There is no noticeable difference in mean size between the two machine-made groups or between the two hand-made groups.

So far as variation in size goes, the difference between the two machine-made groups is not significant at 5%, though the actual value of P , 6.4%, suggests a possible

difference. The difference between the two hand-made groups is significant, however, with $P < 1\%$. Thus the degree of distortion of leucocytes produced in the machine-spread films is more uniform than that in hand-made ones, if the latter are made by more than one observer. When therefore variations in cell size between different films have to be determined, it is advantageous to use the machine for spreading the blood, as the degree of error introduced by accidental technical factors is thereby diminished.

Summary and Conclusions

The paper describes an apparatus designed to eliminate some accidental factors contributing to irregularity in the composition of blood films made on slides. With its aid good films can be produced with very little practice, even by inexperienced workers. Films are characterized by very straight edges, facilitating "battlement" counting, and a relatively short "tail."

An analysis of differential counts performed on hand-made and machine-made films confirmed the previously known fact that in the former, owing to irregularities in the cell distribution, at least 300 cells have to be counted in order to obtain results representative of the total count in the film, or in a series of films made from the same sample of blood. In machine-made films, on the other hand, the distribution was found to resemble a binomial one, for counts as low as 100 leucocytes, thus allowing the easy calculation of the statistical error and of confidence limits. The use of this apparatus saves considerable time in routine differential counting and gives an increase of accuracy for counts of less than 300 leucocytes.

A comparison of the size of leucocytes showed a smaller mean size in machine-made than in hand-made films, and a greater variation in cell size between two groups of slides made by hand by two different observers, than between two corresponding groups made by machine. The apparatus thus produces a more uniform cell distortion than the usual hand method, a fact of some importance where sizes of leucocytes are to be compared in different films.

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