TECHNICAL METHODS

A NEW TYPE OF BLOOD FILM FOR DIFFERENTIAL COUNTS

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Determination of the lymphocyte count is becoming of increasing importance and would undoubtedly be undertaken more frequently, for example in patients under treatment with cortisone or A.C.T.H., if the standard procedure were not so tedious.

Some help is afforded by the use of machine-made blood films as described by Marks, Bailey, and Gunz (1950) because the number of cells which must be counted to attain a given degree of accuracy is smaller than in the case of hand-made films, but the labour involved is still considerable. Enumeration of lymphocytes directly in the haemocytometer has been suggested as an alternative but is, in the writer's experience, difficult and not altogether reliable.

A new type of thick blood "film" has therefore been designed which enables an accurate differential count to be performed rapidly and has the further advantage that a reliable estimate of the standard error can be made.

Routine Procedure

An ordinary draughtsman's ruling pen is prepared by coating the blades with a silicone preparation (Dri-film 9987, American General Electrical Co.) so that their surface becomes non-wettable. The pen is then loaded with a drop of blood and a "film" consisting of one or more lines, each approximately 0.25 mm. wide, is ruled on an ordinary glass slide as shown in Fig. 1. To obtain a uniform and at the same time thick "film" the pen is drawn backwards and forwards four or five times over each line. The slide is left for some hours to dry, or alternatively is dried more rapidly in a warm but not hot oven. It is then stained with Field's stain by the method normally used for the staining of thick blood films for malaria diagnosis. The red cells are all lysed, and, provided care is taken to avoid overstaining, the distinction between polymorphonuclear and mononuclear cells is easily made. The further subdivision of the latter into lymphocytes and monocytes is more difficult, but this, fortunately, is seldom important in the type of work for which the film was designed.

Counting is best done with an oil-immersion objective (1/12 in. or 1/8 in. if available). A start is made by counting all the cells in a narrow strip of width equal to the diameter of the field and running right across the line at some convenient site. The slide is then moved to right or left a distance equal to the diameter of the field and another strip counted. By repeating this procedure all the cells in any desired segment of the line are included.

FIG. 1.—The slide is held in an ordinary slide tray. The photograph has been taken from an unusual angle to show details of the pen, and gives the impression that the latter is held obliquely. In reality, however, it is held almost normally to the plane of the slide.

Owing to the thickness of the film the leucocytes are closely packed, there being in a typical case an average of seven cells per field. A given number of cells may therefore be counted in a much shorter time than would be required with a conventional film. It took the writer, for example, 17 minutes to count 1,000 cells on a line film and an hour to do a battlement count of 1,000 cells on a conventional film from the same individual.

Statistical Investigation of the Cell Distribution

Blood from the finger of a healthy young adult was transferred with a siliconed pipette to the pen and successive lines were then ruled until all the blood had been used ; 7 ml. gave in all eight lines each 6 cm. long.

After the films had been dried and stained every leucocyte in the whole of the first line and in a randomly chosen segment 1 cm. long from each of the other lines was counted, each cell being classified as either a polymorphonuclear or mononuclear cell. A record was made of the number of cells of each type in successive strips of width equal to the field diameter (approximately 0.15 mm.), and from this the number in each successive millimetre or centimetre was obtained. In addition the number of mononuclear cells in each successive 100 total cells was noted. For comparison a total leucocyte count was performed at the same time in a haemocytometer and a conventional film was also made. A differential count was performed on the latter using the standard battlement technique described in a previous paper (Woodruff and Forman, 1950). One thousand cells were counted, the proportion of mononuclear cells in each successive 100 total cells being recorded as before.

In the haemocytometer 327 leucocytes were counted giving a total leucocyte count of 7,670 cells per c.mm. with a standard error of ± 440 cells per c.mm., assuming that shaking of the pipette was sufficient to give the theoretical Poisson distribution. The estimated total count by the line film was 50,705 cells per 7 c.mm. blood, i.e. 7,240 cells per c.mm. There is clearly no significant difference between the two results, from which it may be concluded that all the cells in the line film can be accounted for.

The 10 successive hundred cells in the battlement count gave a mean value of 39.50% mononuclear cells, with a standard deviation of $\pm 1.5\%$. The distribution conformed well to a binomial distribution ($\chi^2_{[9]} = 8.4$, $P \approx 0.5$). This is of interest since Marks *et al.* (1950) found a binomial distribution in a series of counts of 100 cells from machine-made but not from hand-made films. It may be pointed out, however, that the hand-made film used in the present investigation was made and counted by a carefully standardized procedure.

The mononuclear cell count for the 69 hundreds in the first line of the linear film conformed well to a binomial distribution ($\chi^2_{[68]} = 57.8$, $P \approx 0.8$). The 6 cm. of this line were further tested for homogeneity and gave a satisfactory result ($\chi^2_{[5]} = 4.3$, $P \approx 0.5$). The 7 cm. from the other seven lines were also tested for homogeneity and proved satisfactory ($\chi^2_{[6]} = 4.5$, $P > 0.6$) and so did the whole 13 cm. tested together ($\chi^2_{[12]} = 9.6$, $P > 0.6$). Accordingly it was possible to give a mean value for all the readings on the linear film, and this came to $40.82\% \pm 0.41\%$ mononuclear cells.

The difference between the battlement and linear estimate is clearly not significant ($1.32\% \pm 1.61\%$ mononuclear cells).

Summary and Conclusions

This type of film should prove to be of great value when a rapid but accurate estimate is required of the proportion of polymorphonuclear cells on the one hand and of lymphocytes and monocytes together on the other hand. One thousand cells may be counted in about 15 minutes, and since the distribution corresponds closely to the binomial the standard error may be calculated from the following formula:

$$\text{S.E. (expressed as \% of polymorphs or mononuclears)} = \sqrt{\frac{P(100-P)}{n}}$$

where $P = \%$ polymorphs and $n = \text{total number of cells counted}$. Thus, for example, if $P = 50\%$, the S.E. for a count of 1,000 cells is $\pm 1.58\%$. For the same proportion of polymorphs if only 100 cells are counted (taking no more than two minutes) the S.E. is $\pm 5\%$.

In addition to saving time the line type of film saves space. This is particularly the case when several successive counts are required from one individual, as six to eight lines, each representing one count, may be placed on a single slide.

The application of this type of film to the diagnosis of malaria is obvious.

One possible refinement has been considered, namely, the use of a line film equal in width to the diameter of the microscope field, so that the slide need be moved in one direction only. It has been found possible to achieve this for a $1/8$ in. but not for a $1/12$ in. objective. If, therefore, an oil-immersion $1/8$ in. objective is available this modification is worth trying.

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REFERENCES

- Marks, J., Bailey, N. T. J., and Gunz, F. W. (1950). *Journal of Clinical Pathology*, **3**, 168.
Woodruff, M. F. A., and Forman, B. (1950). *Brit. J. exp. Path.*, **31**, 306.