INTERPRETATION OF HISTOGRAM

In order to facilitate the detection of minor variations in cell-volume distribution, which may not be apparent on direct visual examination of the histogram, two indices were elaborated, macrocytic index and microcytic index. The macrocytic index was a ratio of the total numbers of the macrocytes to normocytes and the microcytic index the ratio of microcytes to normocytes. Microcytes were taken as those cells which peaked in windows 5 to 10 inclusive, *i.e.*, less than 72 μ . Normocytes were those cells which peaked in windows 11 to 14 inclusive, *i.e.*, 72 to 100.8 μ . Macrocytes were those cells which peaked in windows 15 and over, *i.e.*, greater than 100.8 μ . The indices were calculated by comparing the sums of the heights of the various peaks in each section. In the 50 normal histograms produced, the microcytic index was never greater than 0.50 and the macrocytic index never greater than 0.75.

To assess reproducibility a bulk dilution of red cells was made and divided into seven aliquots: histograms of each of these were traced and the macrocytic and microcytic indices were calculated (Table).

TABLE

Index	1	2	3	4	5	6	7	Average
Microcytic	0.68	0.68	0.68	0.67	0.64	0.64	0.63	0.66
Macrocytic	0.61	0.62	0.60	0.59	0.63	0.58	0.67	0.61

Figure 2 illustrates the different patterns of histogram obtained by screening a random sample of routine specimens, categorized according to a predominantly microcytic, normal, or macrocytic cell population.

We are indebted to the York 'A' Hospital Management Committee for arranging the purchase of this machine; and to Dr. G. A. C. Summers at whose suggestion the particular model was chosen. Our thanks are also due to members of the laboratory staff for donations of normal blood samples.

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SYMPOSIUM ON IMMUNOASSAY ON CLINICAL CHEMISTRY

The eighth West European Symposium of Clinical Chemistry, on immunology in clinical chemistry, will be held in Newcastle upon Tyne, England, on 28-29 March 1968. Sessions will be arranged on structure and functions of immunoglobulins, serological analysis, radio-immunoassay, and transplantation. For further details please write to the Organizing Secretary: Dr. T. R. C. Boyle, Department of Clinical Biochemistry, Royal Victoria Infirmary, Newcastle upon Tyne, England.

Measurement of red cell diameter by image shearing

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METHOD

A description of the use of the Watson image shearing eyepiece (Wise)¹ in the haematological laboratory may be of interest. This instrument can be used to determine the mean red cell diameter and coefficient of variation in a stained blood film and can also provide a rapid method for screening films for macrocytosis. The Wise employs a dichroic beam splitter which enables the image to be sized by comparison with itself. The object forms red and green images within the eyepiece, which are superimposed in the zero position, but which separate in a vertical direction on rotation of the control knob. To make a measurement, rotation is continued until the images no longer overlap, but just touch edge to edge. A reading taken of the degree of shear required to achieve this, multiplied by a calibration factor previously obtained by observing the stage micrometer, enables the vertical dimension of the object to be measured.

To measure red cell diameters, a 2 mm. oil immersion objective and a $\times 10$ eyepiece are used. A polaroid screen is placed in the condenser light path and rotated to give optimum viewing conditions so as to restore the colour balance of the image pair, which would otherwise be upset by the red colour of the cells in the stained film.

The Wise is set at 7 μ as determined by a preliminary calibration. With this setting the image pairs from red cells, 7 μ in diameter, will be just touching, the pairs from cells greater than 7 μ will be overlapping, while the pairs from cells less than 7 μ will be separated. Two hundred cells are classified in this way. (Cells which are not circular present a difficulty, but can be included in the assessment because the Wise provides an estimate of the vertical diameter which can then be compared mentally with the horizontal and an average diameter assigned.) This procedure is repeated with the Wise set at 8 μ and again at 9 μ (Fig. 1). When 200 cells have been classified at each of three settings the figures for a Price-Jones curve can be worked out (Table I) and analysed in the usual way to give the mean cell diameter and coefficient of variation (Dacie, 1956).

Films showing anisocytosis over a considerable range (as in pernicious anaemia) and those showing microcytosis require modified procedures. When examining films showing considerable anisocytosis at the 9 μ setting, image pairs which overlap are not simply classified as 9.5 μ as in Table I but the degree of overlap is noted and a diameter of 9.5, 10.0, 10.5 etc., is assigned as seems appropriate to the degree of overlap. Similarly at the 7 μ setting, the degree of separation of the image pairs which

¹W. Watson and Son Ltd., Barnet, Herts.
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TABLE I
EXAMPLE OF CONVERSION OF OBSERVATIONS WITH THE WISE TO A PRICE-JONES CURVE

Wise Setting μ	Diameter of Cells μ	No. in Each Group (out of 200)	Price-Jones Curve	
			No. in Each Group (out of 200)	Cell Diameter μ
7	<7	25	25	6.5
	7	104	104	7.0
		129		
8	<8	156	156-129 = 27	7.5
	8	27	27	8.0
	>8	17	17- 9 = 8	8.5
9		9		
		6	6	9.0
	>9	3	3	9.5

fail to touch is noted. With films showing microcytosis, a 6 μ setting is substituted for the 9 μ setting.

Films can be rapidly screened for macrocytosis without determining the mean cell diameter. The Wise is set at 8 μ , 200 cells are examined, and counts are made of the number of cell pairs which just touch (8 μ) and of the number which overlap (> 8 μ). The following criteria have been adopted after study of the results obtained at the more than 8 μ setting with blood films of various types. Definitely macrocytic, 75 cells with diameters > 7.5 μ together with more than 20 cells with diameters > 8 μ ; macrocytosis excluded, less than 50 cells with diameters

crowded should be chosen for the examination. Re-examination of one film where the Wise had given misleading results (film 10 referred to below) suggested that it was too lightly stained for comfortable examination by

FIG. 1. Film from a case of pernicious anaemia with Wise set at 9 μ (image pairs from cells 9 μ in diameter just touch). The red member of each image pair is vertically above the green and appears darker in the monochrome print.

> 7.5 μ together with less than 20 cells with diameters > 8 μ .

The films to be examined should be well stained so that the image pair can be easily seen. In the present investigation it was found that nearly all routine films were suitable though a part of the film where the cells were not

FIG. 2. Mean cell diameter determined by the Wise plotted against mean corpuscular volumes derived from red cell counts carried out on the Coulter counter.

the Wise and ought to have been rejected. It was found that with practice the observations and calculations necessary to determine the mean cell diameter and coefficient of variation in a case of pernicious anaemia could be made in about 30 minutes; with films showing less anisocytosis a shorter time was required.

RESULTS

Films were obtained from normal blood samples and from various types of anaemia and the results obtained using the Wise were compared with the haematological diagnosis (Table II). There was good agreement. Films from 37 of 40 cases of pernicious anaemia were clearly distinguished as macrocytic. Figure 2 shows the relationship between mean cell diameters determined by the Wise and the mean corpuscular volumes derived from red cell counts on the Coulter counter. Since films showing spherocytes or leptocytes were not included, these two determinations can be regarded as alternative methods

TABLE II

NORMAL BLOOD FILMS AND FILMS FROM VARIOUS TYPES OF ANAEMIA MEASURED BY THE WISE¹

Diagnosis	No. of Cases	Mean Cell Diameter (μ)		Coefficient of Variation (%)	
		Mean	Range	Mean	Range (%)
Normal blood	20	7.3	7.0-7.5 (one at 7.9)	5.6	3.6- 7.4
Normochromic normocytic anaemia	20	7.3	6.8-7.4	6.9	2.9- 9.7
Hypochromic anaemia	20	6.7	6.0-7.3	8.7	4.8-12.1
Folic acid deficiency	10	7.6	7.3-8.0	9.3	7.0-14.0
Macrocytic anaemia not due to vitamin deficiency	10	7.8	7.5-8.3	8.3	6.0-11.2
Pernicious anaemia	37 (see note)	8.2	7.6-8.5	9.4	6.3-14.0

¹Films from 40 cases of pernicious anaemia were examined. Anomalous results were obtained with three films which have been excluded from the table but are discussed in the text.

TABLE III

RESULTS WITH PRICE-JONES METHOD COMPARED WITH THE WISE

		Film No.													
		1	2	3	4	5	6	7	8	9	10	11	12	13	14
Mean cell diameter (μ)	Price-Jones	6.5	6.5	6.7	6.9	7.0	7.1	7.1	7.3	7.3	7.7	7.7	8.1	8.6	8.6
	Wise	6.5	6.7	7.2	7.1	7.4	6.9	7.5	7.4	7.1	7.4	8.2	8.1	8.7	8.2
Coefficient of variation (%)	Price-Jones	10.6	8.6	6.0	11.3	6.9	11.5	10.8	6.5	6.6	9.4	11.4	12.7	11.9	7.2
	Wise	9.2	9.0	5.9	11.5	6.9	11.2	11.9	5.5	6.0	9.2	7.2	12.8	9.8	9.2

for the measurement of red cell size (Vaughan and Goddard, 1934). Figure 2 shows that there is reasonably good agreement between the two methods. Table III shows a comparison between the Wise results and a photographic modification of the Price-Jones method.

In three cases of pernicious anaemia the mean cell diameters, as determined by the Wise, were normal although the colour indices and mean corpuscular volumes were high. These films were re-examined by the Price-Jones method (films 4, 6, and 10 of Table III). This showed that in two of the cases the misleading results were inherent in the films while in the third (film 10) the result was partly inherent in the film and partly due to observer error using the Wise.

COMMENT

The Wise used as described is somewhat less accurate than the Price-Jones method and shares with the Price-Jones method the errors inherent in the use of stained films as a source of measurement, difficulties which are discussed by Dacie and Lewis (1963). Cells shrink by 8-16% during the preparation of the film, the apparent diameters can vary in different parts of the film, and it is difficult to assign an average diameter to some of the abnormally shaped cells seen in certain types of anaemia, notably in severe pernicious anaemia. Because of these difficulties the results obtained by the Price-Jones method are hardly ever worth the trouble involved and the method is obsolete. It is generally agreed, however,

that in spite of these difficulties the impression of cell size given by a stained film is usually correct, and a quick and simple method for the verification of that impression by measurement can therefore be justified. Dacie and Lewis commend the use of the eyepiece micrometer as a simple and direct method. The Wise is easier to use than the eyepiece micrometer and the results obtained are likely to be more accurate. In routine cases it will seldom be necessary to proceed beyond the screening test for macrocytosis. The Wise will probably be most useful in a laboratory where the volume of work does not justify the purchase of an electronic blood counting machine and where there is a consequent difficulty in obtaining accurate measurements of the mean corpuscular volume. In such a laboratory, films from cases of macrocytic anaemia turn up intermittently and there is a correspondingly greater need to verify impressions of cell size by measurement.

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