# THE DIFFRACTION PATTERN OF DRY BLOOD SMEARS

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ABSTRACT A theoretical model for dry blood smears is investigated. Circular elements with normally distributed radii and ellipses are considered. The differences in diffraction pattern of sparse and dense smears are explained. The theory is supported by some recordings by means of a simple photoelectric device. A method for calculation of a distribution measure of the radii is suggested.

## INTRODUCTION

If a plane wave of light passes through a plate of glass upon which a number of similar, disc-shaped particles are distributed (e.g. a blood smear), and then through a positive lens, a halo of multicolored concentric rings can be observed around the image of the light source. The diameter of the halo depends upon the diameter of the particles, a fact on which Pijper (1919) bases a method for determining the size of red blood cells. Pijper used a 2.5% blood suspension in a wedge-shaped cuvette, and made the measurement in an area of the suspension layer where the halo appeared most clearly. This seems to have corresponded to a density of about 1 cell per 100 $\mu^2$. White light was used, and the first yellow ring, which appeared brightest, was measured. The diameter of the red cells was accurately estimated by means of the expression

$$\sin \Omega = \lambda/2R_o \tag{1}$$

where $\Omega$ is the angular deviation for the first yellow ring, $\lambda$ is the wavelength of the yellow light, and $2R_o$ is the diameter of the blood cells.

The same formula holds for a linear grating with a grating constant of $2R_o$, but this agreement does not explain the halo phenomenon. From a theoretical point of view it is astonishing that a smear of round cells should behave like a linear grating. As Bergansius remarked (1921), the correct equation for the position of the $n$th diffraction maximum for opaque discs of diameter $2R_o$ is approximately

$$\sin \Omega_n = (n + 0.67)\lambda/2R_o. \tag{2}$$

The constant for the first maximum is thus 1.67 instead of 1.0 as in equation (1). Bergansius noted, however, that in practice the measurements agreed better with Pijper's relation than with equation (2). As an explanation Bergansius assumed that their lens properties caused blood cells to form a grating of luminous points. The distance between these points was said to determine the grating constant. Only if the blood cells were dense could a correct value for their diameters be obtained.

Both these investigations, and other more recent ones, are seriously invalidated by the fact that white light was used to measure the halo phenomenon. As Harnapp and Möbius (1935) remarked, no correlation with any theory at all can be expected under such circumstances, since Newtonian color mixtures of halos of different order displace the position of a color maximum. These authors made photographs of the diffraction patterns in monochromatic light and the photographic negatives were then measured in a densitometer. Like Bergansius, Harnapp and Möbius found that with an increasing number of cells per unit area, i.e. a decreasing mean distance between the cells, lower values of the cell diameter are obtained. However, the effect is not as pronounced as demanded by the theory of Bergansius. Harnapp and Möbius also investigated stained blood cells in the light of the complementary color of the dyestuff and found a diffraction pattern here as well. On the other hand, if the preparation was embedded in a medium with the same refractive index as the blood cells, the halo phenomenon disappeared. The conclusion was that heterogeneity is most important, and absorption less so, for the generation of a halo. According to Harnapp and Möbius the blood cells behave like opaque discs, especially in a dilute suspension.

Verveen (1949) criticized the "opaque disc" model. He found a better agreement between microscope and diffractometric measurements of the diameter when a red cell model consisting of a torus surrounding a concave disc was used. The absorption of light passing through the cell at a certain point depends upon the thickness of the cell at this point. The measurements were performed on stained and unstained erythrocytes in a suspension, by using a simple optical apparatus and monochromatic light for the determination of the diffraction angles.

Harnapp and Möbius also discussed the more general problem of determining the frequency distribution of diameters in an erythrocyte population. They found by investigating pathological smears that the intensity distribution of the diffraction pattern cannot be plotted as a Price-Jones curve. On the assumption that the diameters are normally distributed—sometimes a rather bold approximation—Ponder (1929) earlier suggested a measure, obtained from the intensity distribution of the diffraction pattern, by which the spread of the presumed normal distribution could be determined. This investigation is discussed in some detail below.

The lack of a satisfactory theory for the formation of the halo by blood smears is evident from the investigations quoted. In this paper the halo phenomenon is discussed from a theoretical point of view, and the discussion is limited to dry

smears. The conclusions drawn are illustrated by some measurements of the light distribution in the halo. Finally, the information which can be gained from diffraction analysis is discussed.

## DIFFRACTION PATTERN OF ONE SINGLE ELEMENT

If a particle is 20 or more times larger than the wavelength and has a refractive index considerably greater than that of the surrounding medium, the intensity of the reflected and refracted light in the narrow region around the direction of the incident light can be neglected in comparison to that of the diffracted light (Van de Hulst, 1957). A dry blood smear nearly satisfies these conditions: it is bounded by air on one side, the refractive index of the cells is considerably higher than that of air, and the cells are about 16 times larger than the wavelength if green light is used. The assumption of Harnapp and Möbius that the blood cells can be treated as opaque discs therefore seems reasonable. According to Babinet's principle, the diffraction pattern of an opaque corpuscle is the same as that from a hole, in a black screen, of the same form as the geometric shadow of the particle. Let a parallel beam of light with wave number $k = 2\pi/\lambda$ fall normally on an opaque screen in the $xy$ plane. The screen contains a hole of arbitrary contour. The amplitude of light (apart from a proportionality factor) in the image ($\xi\eta$) plane is then given by

$$u(\xi, \eta) = \iint_S e^{ik(x\xi+y\eta)}\, dx\, dy. \tag{3a}$$

The region of integration, $S$, is the entire $xy$ plane. Two special cases are of interest, ($a$) a circular aperture (disc) and ($b$) an elliptic aperture.

For a circular aperture (case $a$) with radius $R_o$, polar coordinates are a convenience both in the plane of the aperture ($P,\theta$) and in the plane of the diffraction pattern ($\rho,\psi$). Then $x = P\cos\theta$, $y = P\sin\theta$, $\xi = \rho\cos\psi$, $\eta = \rho\sin\psi$, and

$$u(\rho, \psi) = \int_0^{R_o}\int_0^{2\pi} e^{ikP\rho\cos(\theta-\psi)} P\, dP\, d\theta. \tag{3b}$$

The light intensity at $\rho$ is then found to be

$$I(\rho) = [2\pi]^2 R_o^4 [J_1(kR_o\rho)/(kR_o\rho)]^2 \tag{4}$$

where $J_1$ is a Bessel function of the first order. The intensity function has a series of maxima, the positions of which are given approximately by equation (2).

The diffraction pattern of an elliptic corpuscle (case $b$) is also of considerable hematological interest. It may be derived from that of a circular one by a linear transformation of variables (Lommel, as cited by von Laue). If an elliptic element with major and minor axes $mR_o$ and $R_o$ is located with the long axis in the $x$ direction, and the variable $x$ is transformed to $mx$ and $\xi$ is transformed to $\xi/m$, the value of the integral (3a) is unchanged (apart from a factor $m$). The maxima and minima

of the diffraction pattern, which form concentric circles in the case of a circular aperture are deformed to ellipses when the aperture is an ellipse. The eccentricity of the aperture is the same as that of the diffraction pattern. The intensity pattern then depends upon the orientation of the aperture. If $\phi$ is the angle between the major axis of the particle and the direction in which the intensity is recorded, the intensity is given by

$$I(\rho, \phi) = [2\pi]^2 R_o^4 m^2 \{ J_1[kR_o\rho h(\phi)]/[kR_o\rho h(\phi)] \}^2 \tag{5}$$

where

$$h(\phi) = [m^2 \cos^2 \phi + \sin^2 \phi]^{1/2}; \qquad 1 \leqq h(\phi) \leqq m.$$

## DIFFRACTION PATTERN FROM SEVERAL IDENTICAL PARTICLES DISTRIBUTED AT RANDOM IN A PLANE

If $N$ elements are placed with identical orientation in the $xy$ plane, the coordinates of their centers being $x_i, y_i$, the resulting amplitude (apart from a proportionality factor) will be

$$u(\xi, \eta) = \Psi(\xi, \eta) \sum e^{ik(x_i\xi + y_i\eta)} \tag{6}$$

where $\Psi$ gives the diffraction pattern for each element. The intensity $I(\xi,\eta)$ is found by squaring this expression (v. Laue, 1915);

$$I(\xi, \eta) = |\Psi|^2 \sum_i^N \sum_j^N e^{ik[(x_i - x_j)\xi + (y_i - y_j)\eta]}$$

$$|\Psi|^2 \left\{ N + 2 \sum_i^N \sum_j^N \cos k[(x_i - x_j)\xi + (y_i - y_j)\eta] \right\}; \qquad i \neq j \tag{7}$$

$$|\Psi|^2 \, |\Phi|^2$$

If the distribution is random, the sum of the positive and of the negative terms in the double sum tend to the same limit, and the sum will tend to zero. The intensity distribution will on an average be the same as that of a single particle, if the particles are circular, times the number of particles: $I^2 = N \cdot |\Psi_N$.

When the elements are elliptical, they are no longer symmetrical around their centers and the probability that the particle's axis is oriented in $\phi$, $\phi + \Delta\phi$ is proportional to $\Delta\phi$. It can be shown that the average intensity pattern for a random distribution is

$$I_{Av} = 2\pi N m^2 R_o^4 \int_0^{2\pi} \{ J_1[kR_o\rho h(\phi)]/[kR_o\rho h(\phi)] \}^2 \, d\phi \tag{8}$$

The random condition means that a particle falls independently of the position of the other cells. The probability of finding a particle inside a small surface of the size $dS$ is proportional to $dS$. Since the blood cells have a finite size and we demand that the cells cannot be placed on each other, the random condition can only be

fulfilled approximately. The approximation is better in a sparse distribution than in a dense one (Hamaker, 1958; Turner and Eadie, 1957). These authors investigated the number of cells falling in the squares of a Bürker chamber.

The findings of Bergansius and of Harnapp and Möbius, that the number of elements per unit area in the preparation is important for the shape of the diffraction pattern, are therefore not surprising and are also easily verified. The most obvious change in the pattern when blood smears of increasing density are analyzed is that the maxima are displaced outwardly from the central beam. The first-order minimum also deepens (Fig. 1). The difference in position between the first maximum in

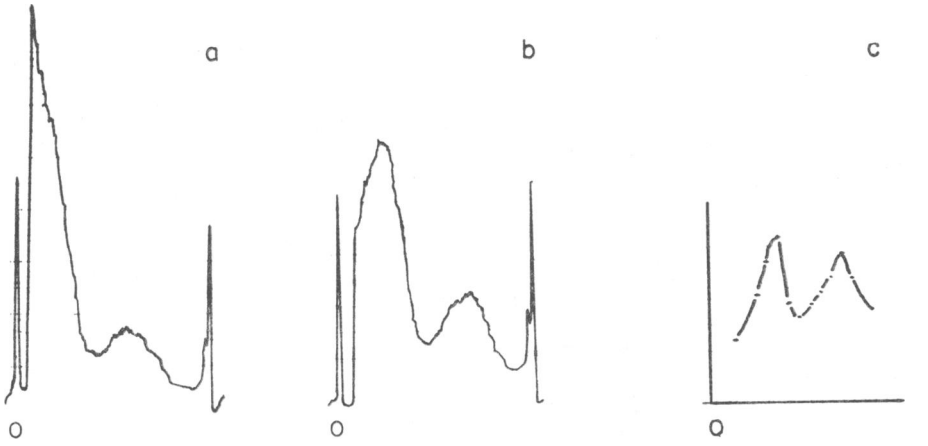


FIGURE 1 Diffraction patterns recorded from smears prepared from the same blood. The ordinate values are relative intensities multiplied by the angular deviation (for *a* and *b*) and the abscissa values are angular deviations. *a*, Sparse smear; *b*, dense smear; *c*, rough outline of the grating function $\Phi^2$, obtained by division of *b* and *a*.

a dense and in a sparse distribution amounts to 15 to 20% of the distance from the zeroth- to the first-order maximum. If the position of the first maximum is measured and introduced in equation (2), a measure of the radius is obtained which is in good agreement with that measured directly under the microscope, but only if the smear is sparse. However, even the sparse smear gives a curve for the intensity distribution which differs strikingly from that represented by equation (4). This equation has minima where the function is zero, whereas the experimental diffraction pattern from a smear show minima of finite intensity.

## RANDOM DISTRIBUTION OF CIRCULAR, NONIDENTICAL, OPAQUE ELEMENTS

The diameter of erythrocytes is usually given as a mean value only. Sometimes a frequency curve is constructed on the basis of measurements of a fairly large number of cells (500 or 1000). Various methods are used in these measurements,

but usually they are all taken in a fixed direction determined by the orientation of the measuring scale in the micrometer eyepiece. The measured values are grouped, and a histogram (often called a Price-Jones curve) is drawn. In normal cases this curve has a shape resembling the gaussian curve, with a standard deviation which is a significant fraction of the mean diameter. If we want to estimate a possible similarity between the diffraction pattern of opaque circular disc and that of a blood cell population, it is evidently necessary to take the width of the distribution into account. Our calculation of the theoretically expected diffraction pattern has been made on the assumption that the diameters of the particles are normally distributed. Ponder (1929) made the same assumption; however, he erroneously used the amplitude instead of the squared amplitude for the intensity pattern from a circular opening. The frequency function for cells of radius $R$ is given by

$$f(R) = [1/(\sigma\sqrt{2\pi})]e^{-(R-R_o)^2/2\sigma^2}, \tag{9}$$

where $\sigma$ is the standard deviation and $R_o$ is the mean radius. The intensity distribution in the pattern is obtained from equations (4) and (9):

$$I_{Av}(\rho) = [(2\pi)^2 N/(\sigma\sqrt{2\pi})] \int_0^\infty R^4[J_1(kR\rho)/(kR\rho)]^2 e^{-(R-R_o)^2/2\sigma^2}\, dR. \tag{10}$$

If the parameter values $R_o$ and $k$ are fixed, we obtain, for every value introduced, a curve which indicates how the intensity depends on the angle of deflection. This calculation has been performed for $2R_o = 7.5\ \mu$, $k = 2\pi/\lambda$, and $\lambda = 5500$ A. Values of $\sigma$ from 0 to 0.20 $R_o$, with intervals of $0.02R_o$, have been used. The intensity falls very rapidly with an increased angle of deflection. With the photoelectric equipment used in the present investigation for recording the diffraction pattern, a wedge-shaped slit was placed before the phototube in such a way that the effective slit opening increases in proportion to the distance of the slit from the zero position (angle of deflection, $\Omega$). The values calculated from equation (10) have therefore been multiplied by a factor $kR_o\rho$ to admit a direct comparison between the blood swear curves and the theoretical values. These corrected values have been used in drawing the family of curves in Fig. 2.

The introduction of a diameter distribution of the circular discs of the model has certain obvious consequences for the shape of the diffraction pattern.

1. When $\sigma = 0$, i.e. when all discs are identical, the intensity takes the value zero in a series of minima. With increasing values of $\sigma$, i.e. a more heterogeneous distribution, the maximum values decrease and the minima increase, and the undulations tend to be leveled out. At sufficiently high values, these undulations are completely effaced. The interdependence of the measure of dispersion and the quotient of the intensity at the first minimum to that at the first maximum is seen in Fig. 3.

2. With increasing $\sigma$, the maxima are displaced increasingly toward lower values of $\rho$. The displacement as a function of the measure of dispersion is drawn in Fig. 4. A careful determination of the mean diameter of the particles implies

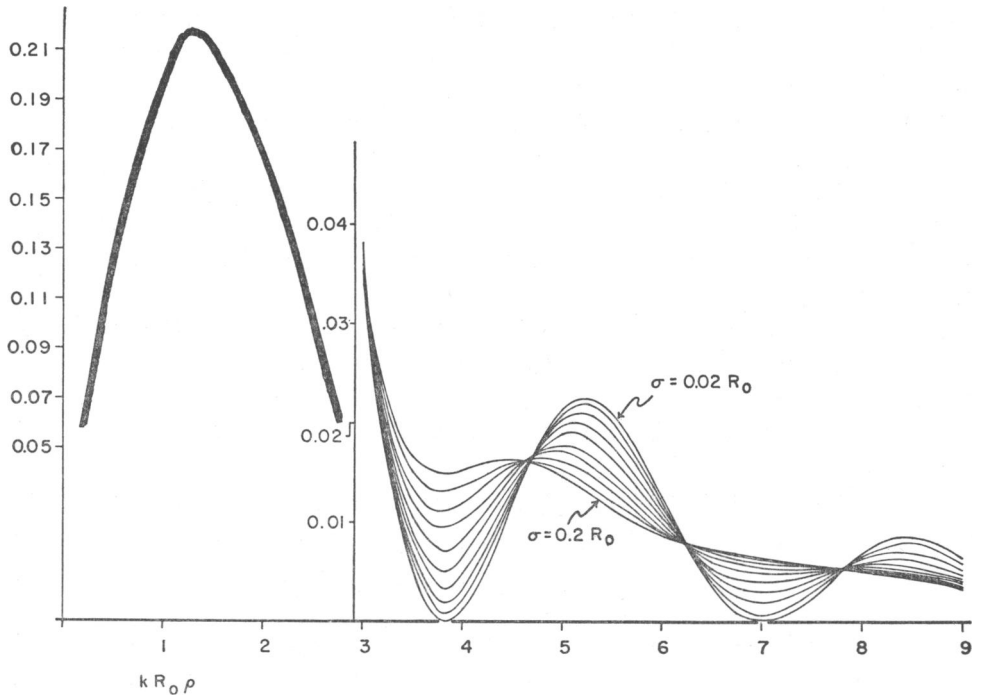


FIGURE 2 Calculated intensity patterns for populations of circular elements with different dispersion of radii. The ordinate values are given by equation (10) multiplied by the abscissa value ($kR_o\rho$).

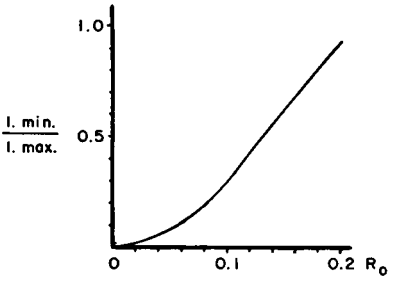


FIGURE 3 The ratio between the intensity of the first minimum to that of the first maximum (ordinate axis) vs. the dispersion measure (abscissa).

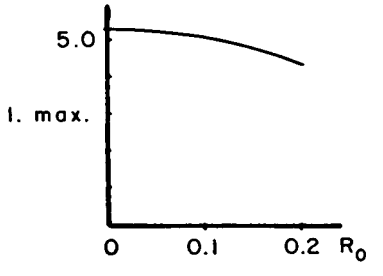


FIGURE 4 Position of the first maximum as a function of the dispersion. The ordinate values are $kR_o\rho$ values from Fig. 2, and the abscissa values are in terms of the standard deviation, $\sigma$.

that references made to the dispersion of the population, and a correction is made for this. The displacement is caused by the factor $R^4$ under the integral sign in equation (10), which stresses the weight of the large particles of the population.

The measure suggested by Ponder gives an impression of the homogeneity of the cell population, but the question arises whether this satisfies the hematologist who is interested also in the skewness, presence of double peaks, etc., in the frequency distribution. Accordingly, we wish to investigate the possibility of calculating something like a Price-Jones curve from the observed intensity distribution of the diffraction pattern.

Instead of the normal distribution of circular elements (equation (9)) we introduce an arbitrary frequency function $f(R)$, and obtain

$$I_{A_v}(\rho) = [(2\pi)^2 N] \int_0^\infty R^4[J_1(kR\rho)/(kR\rho)]^2 f(R)\, dR \tag{11}$$

where $\int_0^\infty f(R)\, dR = 1$. $I_{A_v}(\rho)$ is recorded as a curve or as a series of values. To obtain an approximate solution for $f(R)$ from the integral equation (11) we rewrite it as a sum:

$$I(x_i) = \sum R_j^4[J_1(R_j x_i)/(R_j x_i)]^2 \hat{f}(R_j)\, \Delta R \tag{12}$$

where $I(x_1), I(x_2), \cdots I(x_n)$ are measured relative intensity values at $x_1, x_2, \cdots x_n$, and $\hat{f}(R_j)$ values are to be determined for a number of values of the radii $(R_j)$. In matrix notation we have $I = \alpha \cdot \hat{f}$, where $|\alpha| \neq 0$ and the elements of $\alpha$ are

$$a_{ij} = R_j^4[J_1(R_j x_i)/(R_j x_i)]^2. \tag{13}$$

Then $F = \alpha^{-1} I$. The elements of $\alpha^{-1}$ need be computed only once. The matrix and the series of intensity values are the bases of a digital computer program from which the values of $f$ are calculated in a few seconds. However, it will be necessary to obtain diffraction intensities of very high accuracy in order to actually take advantage of this theoretical possibility.

Consequently, if the elements are circular, the frequency distribution of diameters (i.e. the Price-Jones curve, by measurements in a fixed direction as described above) can be calculated from the intensity values of the diffraction pattern. If the pattern from noncircular elements is subjected to the same calculation, the diameter distribution is not obtained. This can easily be demonstrated for identical ellipses distributed and oriented at random.

We introduce two new variables into equation (8) which describes the pattern made by elliptical elements:

$$R = R_o h(\phi); \qquad R' = dR/d\phi = R_o h'(\phi)$$

so that

$$\int_0^{2\pi} d\phi = 4 \int_0^{\pi/2} d\phi = 4 \int_{R_o}^{mR_o} (-1/R')\, dR = 2\pi.$$

Equation (8) then becomes

$$I_{Av} = 8\pi N m^2 R_o^4 \int_{R_o}^{mR_o} [J_1(kR\rho)/(kR\rho)]^2(-1/R')\, dR \qquad (14)$$

where

$$1/R' = -R/[(m^2R_o^2 - R^2)(R^2 - R_o^2)]^{1/2}.$$

By replacing $f(R)$ of equation (11) by a constant times $R_o^4/R^4\,R'$ we obtain the intensity pattern of equation (14). Thus there exists a population of circular elements with the same diffraction pattern as that from the ellipses. However, the radius frequency distribution of the Price-Jones curve that would be obtained by measuring a smear of ellipses differs from the frequency function for a population of circular elements with the same diffraction pattern.

The function $f$ (the frequency distribution of radii in the equivalent circular smear) is probably as instructive as the Price-Jones curve. In practice the difference between the Price-Jones curve and the function $f$ is probably small, since even in highly pathological smears circular or only slightly elliptic forms dominate. It may therefore turn out to be a convenient practical procedure to measure the intensity pattern of a sparse smear in an accurate diffraction apparatus. The function $\hat{f}$ is then obtained if the data is of sufficient accuracy.

## DENSE DISTRIBUTIONS

The denser the smears the more cells will be in contact. In a smear prepared *lege artis*, we may assume that the cells do not deform each other at the contact points. Thus the reason for diffraction pattern change as the smears become denser must be attributed solely to decreasing similarity to a Poisson distribution. In equation (7), $\Phi$ describes the arrangement of the elements. Although it is a constant in the random distribution, its value varies with $f(\rho)$ in the more dense smears. Attractive or repulsive forces of unknown strength between the cells may influence their arrangement. In general, therefore, it is highly hazardous to try to deduce a statistical model from which the function $\Phi$ could be determined as a function of the cell density only. However, for the extreme and ideal case where the cells are identical and packed as closely as possible, the diffraction pattern can easily be predicted. We then have a crystalline structure in two dimensions (Fig. 5). The first three maxima will appear at the distances $(\lambda/2R_o)(2/\sqrt{3})$, $(\lambda/2R_o)(2)$, and $(\lambda/2R_o)(4/\sqrt{3})$ from the central beam. If, then, the smear is considered to consist of smaller groups with this structure, oriented at random, a circularly symmetrical pattern can be expected with its maxima falling at the distances mentioned. For the treatment of less extreme and idealized conditions, the following model is suggested. Let the smear be made up of small areas, each with a nearly crystalline structure. In each of the areas the cells are placed at $\alpha_o$, $T + \alpha_1$, $2T + \alpha_2$, $\cdots$ $nT + \alpha_n$, where $T$ is the mean distance between the cells in a row and $\alpha_i$ is a stochastic variable distributed according to some frequency function. A model

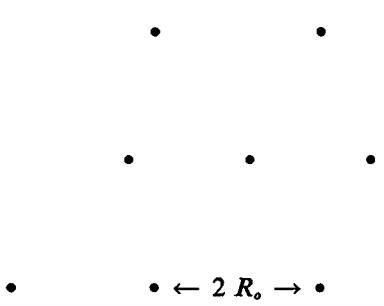


FIGURE 5 Crystalline structure representing a maximally dense smear of identical elements of radius $R_o$.

of this kind has been treated for the one-dimensional case by MacFarlane (1949) and by Fortet (1954). Like the extreme case, this one will have maxima, the positions of which are determined by the mean distance $T$. With increasing freedom for the positions of the elements, the random distribution is approached, i.e., the maxima of the function decrease and the function $\Phi$ approaches a constant value of $\sqrt{N}$.

Why the pattern changes with increasing cell density is now easily understood. In the sparse smear the single-disc pattern dominates and the first maximum is found at $1.67(\lambda/2R_o)$. In the dense preparation this function is multiplied by the grating function which has maxima at $\rho$ equal to $(\lambda/2R_o)(2/\sqrt{3})$, $(\lambda/2R_o)(2)$, etc. A maximum is found near $2.0(\lambda/2R_o)$ which is also approximately the position of the second maximum of a linear grating (equation (1)) with a grating constant of $2R_o$. In densities between these extremes a maximum is found between these two values.

In a very rough way this is illustrated in Fig. 1, where we have a pattern from a very sparse smear and a pattern from a dense one. We get an idea of the grating function $\Phi$ by dividing the pattern from the dense smear by that from the sparse one.

## CONCLUSIONS

The experiments with dry blood smears have given results which are in good agreement with the hypothesis that the cells behave like opaque discs.

In very sparse preparations the cells can be considered to be distributed at random. These preparations are well suited for measurements of the mean cell diameter. A procedure is suggested by means of which a function can be computed with informative value similar to that of a Price-Jones curve.

Dense preparations may possibly afford some sort of information on the mode of arrangement of the cells. The cell density per unit area as a function of the position of the maximum might perhaps tell us something about abnormal tendencies to adherence between the cells. The formula for determining the approximate cell diameter in a dense smear cannot be used for a sparse smear.

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