beneficial effect at all. Intravenous injections of eusol in doses of 50 c.cm. (double strength in distilled water) was tried in one case, and appeared to have some influence in modifying the fever.

In conclusion we would like to acknowledge our indebtedness to Dr. J. T. Duncan of the London School of Hygiene and Tropical Medicine, and Messrs. F. W. Foster and W. J. Muggleton for their kind assistance with the laboratory work undertaken in connexion with the above series of cases.

AN IMPROVED DIFFRACTION METHOD FOR DIAGNOSING AND FOLLOWING THE COURSE OF PERNICIOUS AND OTHER ANAEMIAS.*

BY

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Principle of Method.

WHEN a beam of white light is sent through a blood smear it is broken up (diffracted) by the blood cells into the spectral colours. From the distribution of the colours the mean diameter, the degree and quality of anisocytosis, and the degree of poikilocytosis of the red cells can be determined. The application of the method is simpler than the description. No mathematics are required to read Figs. 3 to 11, where the half-circles illustrate the diffraction spectra exactly as they appear on the screen of the apparatus, two at a time, thus facilitating comparison and reading of results. "Diffraction method" and "diffraction micrometer" are suggested as suitable names.†

Need for Method.

The increasing incidence in many countries of Addison's (pernicious) anaemia,⁴ together with Minot and Murphy's discovery of the liver treatment,⁵ has led to the publication of numerous papers. A curious feature of this literature is the scantiness or absence of numerical data on the diameter of the red blood cells, although the correctness of Hurst's statement,⁶ "the most characteristic features of the blood in Addison's anaemia are the anisocytosis and the increase in the average size of the red cells," is at the same time very generally admitted. Red cell measurements evidently are still universally regarded as "research," and not as "routine." This is due to the lack of a suitable method. Direct microscopic measurement,⁷ and photographic methods,⁸ are manifestly too laborious.

Advantages of Method.

In its present form the advantages of the method are:

1. The only material required is a properly made, unfixed, unstained blood smear. Such smears remain serviceable for years, thus allowing dispatch over long distances, and storage for future reference. Fixed and stained smears are less suitable.
2. The method expresses in tenths of microns the mean diameter of at least one million red cells.
3. The method expresses in tenths of microns the diameter of both the largest and the smallest cells which are present in the smear in considerable number. In other words, it gives the degree and quality of the anisocytosis present. The information thus gained is not sufficient to construct a frequency curve, but ample for practical purposes, as it indicates the essential features of the curve.
4. The method gives the degree of poikilocytosis present in the blood smear.
5. All these determinations together do not take more time than a haemoglobin estimation.

* Part of the apparatus used in working out the diffraction method was bought by aid of a grant from the Union Government Research Grant Board.

† It has repeatedly been brought to my notice that Thomas Young (1773-1829) employed the identical principle, first for the measurement of wool fibres (wool=erion), and later of red blood cells. I cannot regard this as a reason to call my apparatus an erionometer, as Emmons¹ has suggested. When, in 1918, I discovered that bacterial colonies can produce diffraction spectra, I started using this phenomenon for measuring bacteria.² From this beginning I developed my diffraction method of measuring red cells³ in complete ignorance of Thomas Young's work.

6. The cost of the apparatus is moderate.

7. The method allows the direct comparison of the essential characteristics, mentioned above, of any two blood smears. This is helpful in following the course of a disease. (See Figs. 9 to 11.)

As I am dealing with practical issues only, I shall not go into the controversy about the effect of drying upon the diameter of the cells, nor discuss the problem whether there is such a thing as "the exact diameter" of a red cell.

Theory of Method.

The thin end of a properly made blood smear can be regarded as a diffraction-grating, the cells being opaque and the plasma transparent. A beam of white light passing at right angles through such a smear is diffracted at the edges of the cells, and the resulting light waves, collected by a lens placed behind the blood smear, will produce a series of concentric circular spectra in the focal plane of the lens. The size of these spectra is a function of the diameter of the red cells of the smear, the white cells being too few to count.

For practical purposes only the innermost of the circular spectra need be considered, as being the brightest. The formula expressing the relationship between the various factors, the derivation of which can be found in textbooks on physics, is:

$$d = \frac{1.7\lambda \sqrt{f^2 + r^2}}{r}$$

in which (d) is the diameter of the red cells, (f) the focal distance of the lens used, (r) the radius of the coloured circle in the (innermost) spectrum, and (λ) the corresponding wave-length. The formula shows that the diameter of the cells can be calculated from the radius of any coloured circle, that the diameter of the coloured circles and the diameter of the cells vary inversely, and that a lens with a longer focal distance will produce larger spectra.

So far the matter has been treated as if the elements of the diffraction-grating (the red cells) were all of equal diameter. The diameters of the red cells of a healthy person, of course, follow the curve of normal variation. Therefore, assuming that the mean diameter in such a case is 7.3μ , there will be more cells of this diameter than of any other, but there will also be an ever-decreasing number of cells deviating more and more, in opposite directions, from this mean value. The spectrum produced by the cells measuring 7.3μ will naturally be the brightest, these cells being the most numerous. On this spectrum will be superimposed the weaker spectra belonging to the other, smaller and larger, cells. In Fig. 1 are drawn to scale, as calculated from the formula, the spectra thrown by cells measuring 7.3 , 6.7 , and 7.9μ . Intermediate cells will, of course, throw intermediate spectra. The drawing shows that all these superimposed spectra will practically not affect the position of the bright yellow circle produced by cells measuring 7.3μ . They will make the whole yellow circle broader, and the superimposed red and green will dilute the colour. This means that the brightest yellow circle will remain the expression of the mean diameter of the red cells, and that from that yellow circle the mean diameter can be calculated (wave-length 0.59μ).

From Fig. 1 it is also obvious that the innermost violet circle always is the expression of the largest cells present in a blood smear in sufficient number to produce visible colours, and that similarly the outermost red circle represents the smallest cells which are present in the blood

FIG. 1.

smear in similar number. By determining the points where the violet and red colours vanish, and substituting these radii and the corresponding wave-lengths (for red 0.70μ , and for violet 0.40μ) in the formula, both the diameter of the "smallest" and the "largest" cells present in the blood smear in considerable number can be calculated.

The method misses the very largest and the very smallest cells, because they are too few to produce a visible effect. For the same reason direct microscopic measurement does not achieve much more in this respect.

The advantages of direct microscopic measurement lie in the construction of a frequency curve. The important features of such a curve are the position of the top and

the shape of the curve (narrow or wide, symmetrical or not). From the above it is evident that, generally speaking, the width of the spectrum corresponds to the width of the curve, that the position of the top of the curve is given

by the position of the yellow circle, and that the relative positions of the extreme red and innermost violet circle with regard to the yellow circle serve to show whether the curve is symmetrical or not. Healthy people by this method usually show a symmetrical anisocytosis—such as mean diameter 7.3μ , "largest" cells 7.9μ , "smallest" cells 6.7μ .

For estimating the degree of poikilocytosis it must be remembered that the rounder the cells are the better the grating will be. Poikilocytosis thus affects the brilliancy of

FIG. 2.

FIG. 3.

FIG. 4.

FIG. 5.

FIG. 6.

FIG. 7.

FIG. 8.

FIG. 3.—Typical small spectrum of subacute combined degeneration of spinal cord. Spectrum not proportionally narrower, indicating more anisocytosis than normal, and towards small side (10.6, 9.8, and 8.7). No poikilocytosis. Haemoglobin 70 per cent., red cells 3.5 million.

FIG. 4.—Typical small spectrum of an early case of Addison's pernicious anaemia. Little anisocytosis, asymmetrical, towards small side. Some poikilocytosis. Haemoglobin 34 per cent., red cells 2.7 million.

FIG. 5.—Late case of Addison's pernicious anaemia. Small spectrum, very marked anisocytosis, symmetrical. Marked poikilocytosis. Haemoglobin 20 per cent., red cells 3.6 million.

FIGS. 6 AND 7.—Typical broad spectra of secondary anaemia. The heavier the anaemia, the broader the spectrum. Late syphilis often gives a spectrum like Fig. 7. Anisocytosis very marked, asymmetrical, towards large side. Often in such cases poikilocytosis not marked, and mean diameter not much changed.

FIG. 8.—Strikingly large spectrum, diameters of cells very small, as typical for familial haemolytic ictero-anaemia, although cell volume may be large. Little anisocytosis, symmetrical. Poikilocytosis present. Hereditary element traced in abnormally small cells in relatives on father's side, in father, and in brothers and sisters; mother's cells normal. Similar investigation seems indicated in relatives of patients of Addison's pernicious anaemia, to throw light on question of heredity.¹⁰

FIG. 9.

FIG. 10.

FIG. 11.

FIGS. 9 TO 11.—These three figures illustrate the improvement brought about through liver diet in a patient with Addison's pernicious anaemia. Spectra not compared with standard, but

with other smears from the same patient. The haemoglobin rose from 32 to 80 per cent., and the red cells from 1.4 to 5.2 million during the same period.

the colours, and also the number of concentric spectra which become visible round the bright first one. From this the poikilocytosis can readily be estimated, especially as the method permits a direct comparison with a "standard" (Figs. 3 to 11). I need not detail why the method is not absolutely correct. Fair comparison with other methods shows that its advantages outweigh its inexactitudes.

Technique of Method.

A beam, about 6 cm. broad, of parallel light rays, from the sun, or from an artificial source (arc or pointolite) with condenser, is directed towards the apparatus (Fig. 2). Through two square holes, of 1.5 cm., in the screen "A" it reaches the thin ends of blood smears on the microscope slides "B" and "C," which are fixed to the front of the box "D" by clamps. The box is of wood or metal, 25 cm. high and 40 cm. long; the back is a ground glass plate "G," of 25 by 25 cm., with its ground surface towards the light. In front, in the centre, is the lens "E," a simple lens, of 6 cm. diameter and 40 cm. focal length, plano-convex, such as may be bought from an optician for a few shillings. The partition "F" is exactly in the middle, in close apposition to the lens and the ground glass.

It is preferable to have the apparatus in a dark room, and admit the beam through a hole in the wall; if this is not possible other means must be found to exclude all foreign light from the ground glass. It is a help if a disc of black cardboard is pasted over the centre of the ground glass, as the white light which is concentrated there is dazzling, and the centre of the disc can at the same time serve as a starting point for the measurement of radii.

As the partition "F" cuts off one-half of either set of spectra, the ground glass will show two sets of coloured half-circles in spectral colours, separated by a thin black line, the shadow of the partition. Their actual appearance is illustrated by Figs. 3 to 11. The three half-circles in each case represent the position of the extreme red, the bright yellow, and the innermost violet circle of the innermost spectrum, as indicated by the letters "r," "y," and "v."

A "standard" blood smear is obtained by collecting blood smears from "healthy" persons, comparing their diffraction spectra by using them in the apparatus two by two, and picking out one which gives medium values for the radius of the yellow circle, and minimum value for anisocytosis (=broadness of spectrum). It will be found that minor differences in these respects occur between individuals. These differences remain constant for long periods, and perhaps for ever, but intercurrent diseases may at least temporarily affect them. The meaning of these differences, their bearing on blood groups, their

pathological significance, and other points in this connexion (day and night changes, effects of exertion), I shall treat in a later paper.

For the chosen standard smear the radii of the yellow, innermost violet, and extreme red circles are then carefully once and for all determined with a pair of compasses. With my lens, which has a focal distance of 40.4 cm., they measure respectively 56, 35, and 73 mm. From these values the formula gives for the mean diameter 7.3μ , for the largest cells 7.9μ , and for the smallest cells 6.7μ .

In determining the radius of the extreme red circle, it must be borne in mind that, as the second spectrum overlaps the first (or innermost) spectrum to a certain extent, the extreme red circle borders on the green circle of the second spectrum. Together these two colours produce a greyish zone, and the extreme red must be localized there where this grey zone ends, and the green appears quite pure. In localizing the innermost violet one must choose the spot where purple fades into yellow.

The standard radii being known, the radii belonging to any blood smear can easily be estimated by direct comparison, as shown by Figs. 3 to 8. Where the differences are small, the direct comparison makes them appear quite obvious, and for large differences an error of a millimetre in estimating the difference is not important. For accurate work compasses are useful. The values obtained for the radii can be converted into cell-diameters by calculation with the formula, but, of course, it saves time if from the formula a table or a curve is prepared from which the cell-diameters belonging to any radius can just be read off.

It is not always necessary to perform measurements and calculations, as often a glance at the spectra gives sufficient information (see Figs. 3 to 8) to make or exclude a particular diagnosis.

Application of Method.

The method has now been used by me on hundreds of smears, and its diagnostic results have been checked by other methods and observations. It has cleared up many difficult cases. In suspected cases of pernicious anaemia where it gave a typical secondary anaemia spectrum further examination of the patient has revealed early malignancy or duodenal ulcer. It has become an essential routine method, and as such has occasionally given a dramatic diagnosis in unsuspected cases of Addison's pernicious anaemia, aplastic anaemia, subacute combined degeneration of the spinal cord, and familial haemolytic ictero-anaemia, which otherwise might easily have escaped notice.

Note.—Figs. 3 to 8 show the differences between the standard and certain typical pathological spectra observed by me. At the bottom of Figs. 3 to 11 the small filled-in black circles represent, drawn to scale, the cells of mean diameter, the "largest"

and the "smallest" cells, as calculated from the radii of the half-circles shown. The diameters of these cells are given in microns.

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LIVER TREATMENT OF PERNICIOUS ANAEMIA:

ITS EFFECT ON THE POLYNUCLEAR LEUCOCYTE COUNT
OF COOKE.

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IN the course of routine blood examinations of the patients of this hospital, consisting of the polynuclear count of Cooke and the Schilling haemogram, it occurred to me to investigate the influence of liver treatment on the polynuclear count in a case of pernicious anaemia which was to undergo that treatment.

The polynuclear count of Cooke is simply a modification of the original Arneth count. The original Arneth count was far too complicated, and as a direct result of this we have two modifications in clinical use at the present day: the polynuclear count described by Cooke and Ponder¹ in 1927, which most closely resembles the Arneth count, and the Schilling haemogram,² which divides the neutrophil leucocytes into those with one lobe to the nucleus, including in this the myelocyte and the metamyelocyte, and those cells with more than one division to the nucleus. In this study we are only concerned with the polynuclear count. This gives a much more delicate picture of the leucocytes than either the Schilling haemogram or a total white count plus an ordinary differential one.³ The Schilling haemogram does not indicate any abnormality in pernicious anaemia apart from an inconstant lymphocytosis.

In the polynuclear count as described by Cooke and Ponder¹ the percentage of leucocytes with one lobe to the nucleus is multiplied by one, the percentage with two lobes by two, and so on up to the percentage with five or more, which is multiplied by five. The numbers obtained added together and divided by 100 give the weighted mean number of lobes of the nucleus per leucocyte. The average number in each group in health is about—

I.	II.	III.	IV.	V.
10	25	47	16	2

This gives a normal weighted mean of 2.75. The average varies slightly from individual to individual and with individual workers. Arneth's mean is normally given as 2.76, and Cooke in 1925⁴ gave his normal as 2.70. My own mean, taken from members of the staff who are healthy, is 2.65. The normal mean can fairly definitely be taken as lying between 2.50 and 2.90. The first two groups of the count (I and II) are spoken of as the left-handed columns, and groups III, IV, and V as the right-handed columns. The numbers in the two groups I and II added together constitute the left hand, and III, IV, and V the right hand. I have divided the right hand by the left hand

and called this the index of right-handedness (R.H.) in tables. Bushnell and Treuholtz⁵ suggested adding together groups I and II and half of group III. This is referred to in the tables as B.T.

Naegeli⁶ described a pronounced hypersegmentation of the neutrophil nucleus in pernicious anaemia. Arneth⁷ in 1920 described the "Rechtsverschiebung" or shift to the right which accompanies the neutropenia characteristic of pernicious anaemia. Cooke⁴ in 1925 showed that in uncomplicated pernicious anaemia there was an increase in the number of cells in groups IV and V and a decrease in groups I and II. In group V are those cells with five or more lobes to the nucleus; in pernicious anaemia we often find cells with seven or eight lobes, and in the case of the macropolycyte⁸ there may be ten to fourteen lobes. This means, of course, that the weighted mean and the index of right-handedness are both high. Jedlička and Béránek⁹ report the neutrophil picture in 36 cases of pernicious anaemia. There is nearly always leucopenia, although there are cases with neutrophil leucocytosis of long duration. These authors found an increase of the number of cells with more than three lobes to the nucleus, but also a shift to the left—that is, a dissociation of the blood picture. They consider this dissociation of the blood picture to the right as well as to the left a characteristic of pernicious anaemia. However, Arneth¹⁰ has subsequently demonstrated that this dissociation to the right and to the left also occurs in lymphatic leukaemia. Jedlička and Béránek point out that this dissociation is most marked at the height of the disease and may disappear during remission. Arneth had previously suggested that the myelocytes observed by some authors are really erythroblasts, so accounting for a certain number of cells in group I. Krjukoff¹¹ describes a similar dissociation in sprue—the appearance of myelocytes together with an increase in the number of hypersegmented forms. This will be referred to later. Whitby¹² describes how in two out of four cases treated with liver the relative lymphocytosis disappeared and the differential count became normal apart from the eosinophilia. He did not describe any changes in the neutrophils. Other writers in the *Lancet* inquiry into the effects of liver treatment¹³ show usually a return to the normal number of leucocytes, but give no detail of the neutrophil picture.

Cooke gives the following numbers of cells per group in his cases of pernicious anaemia. The respective indices are given in the last three columns.

Case.	Group.					Weighted Mean.	B.T.	R.H.
	I.	II.	III.	IV.	V.			
1	11	24	49	15	1	2.71	53.5	1.85
2	6	20	39	22	11	3.08	45.5	2.25
3	6	27	40	22	5	2.93	47.0	2.03
4	7	20	44	24	5	3.00	49.0	2.70
5	7	16	29	39	9	3.27	37.5	3.35
6	12	31	26	23	8	2.78	59.0	1.17
7	20	31	17	16	16	2.77	59.5	0.96
8	16	22	32	16	14	2.90	54.0	1.63
9	10	15	38	24	13	3.05	45.0	3.00
10	6	20	42	20	12	3.12	47.0	2.85

Of these, Cases 7 and 8 both show a dissociation of the count and Case 6 to a lesser degree. Our case before liver treatment showed—

Group I	...	...	...	...	...	...	1
" II	...	...	...	...	...	...	16
" III	...	...	...	...	...	...	39
" IV	...	...	...	...	...	...	32
" V	...	...	...	...	...	...	11
Weighted mean	...	...	...	...	...	...	3.33
B.T.	...	...	...	...	...	...	36.5
R.H.	...	...	...	...	...	...	4.58

Possibly Cases 1, 6, and 7 of Cooke's series had some inter-current infection or hidden septic focus causing the low weighted mean.