Studies on the Diffractometric Measurement of the Diameter of Erythrocytes.

III. An improvement of Haden-Hausser's erythrocytometer by means of monochromatic light.

By

Kōjirō Matsuda.

(松田幸次郎)

(*Department of Physiology, Tokyo Imperial University.*

Department of Medicine, St. Luke's Hospital, Tokyo.)

INTRODUCTION.

In the previous reports^{2,3)}, I have shown that the accuracy of Haden-Hausser's erythrocytometer in measuring the mean diameter of erythrocytes in cases of blood with pathologically marked anisocytosis is not as satisfactory as in cases of normal blood. Inasmuch as the cases of such pathological blood are the very cases in which we want to know the mean red cell diameter easily and quickly from the practical standpoint, it is very desirable to improve the accuracy of this diffractometer without detracting its advantages such as easy manipulation, low cost etc. In the course of experiment with the diffractometer, I noticed that one of the causes of large error may be the overlapping of different spectral rings which have been broadened and obscured by marked anisocytosis, that is, as the result of the overlapping the true position of the maximum intensity of any color may shift and appear in a false position. This overlapping can logically be avoided if one uses a monochromatic light as the source. Among many investigators who measured diffractometrically the diameter of erythrocytes only Allen and Ponder¹⁾ noticed the necessity of the monochromatic light. I replaced the light source of Haden-Hausser's erythrocytometer with a monochromatic bulb and obtained satisfactory results as shown in the following pages.

METHOD.

The base plate of Haden-Hausser's erythrocytometer with the electric bulb on it was removed and the remaining part consisting of the double cylinders was put upon a glass plate on a wooden box, in which a special electric bulb "Mazda's Natrium Lamp" manufactured by The Tokyo Shibaura Denki Co. was placed. The yellow light from this bulb was made to pass through a filter "Mazda V-A1" and was focussed by condensing lenses to a point about 1.5 cm below the middle of the diaphragm (Fig. 1). The light after it has passed the filter has the wave length of $5,890-96\text{\AA}$. As yellow is the most luminous among all colors, it may be most convenient for observation of its intensity.

Fig. 1. Diagram showing the modified construction of the apparatus.

- Y: Mazda's natrium lamp.
C: Condensing lenses.
F: Filter, Mazda V-A1.
G: Glass plate.

The blood specimen is placed on the outer cylinder as in the previous experiments, and when it is viewed from above a very bright yellow light was observed in the center, and concentrically to that a dark and a yellow rings arrange themselves towards the periphery as it would be expected from the theory of diffraction. Among the pin-holes of the diaphragm, the inner set of four holes can be most distinctly seen as small points, especially when these holes are made to correspond to the middle of the dark ring by moving the external cylinder. The procedure of sliding the outer cylinder up and down is quite the same as before.

The same blood smears were used as in the previous experiments; 12 smears were selected at random from the specimens without patho-

logical anisocytosis, 13 smears from the specimens with pathological anisocytosis, all stained with Giemsa's solution and mounted in saline. The mean diameter in μ had been measured by the direct method in the previous experiments^{2,3}.

The diffractometry consists in taking the reading from the scale on the hand wheel after the position of the maximum darkness of the first dark band is made to correspond to the inner set of holes. Each time the average of 5 readings was taken. These 5 readings usually differ no more than 0.2 from each other.

RESULT AND DISCUSSION.

The result is presented in Table 1 and Fig. 2. The "Number of specimen" indicates the same specimen used in the previous reports^{2,3}. The straight line in the figure was obtained by the least square method as a formula $y=ax$, where y = the value in μ obtained by the direct measurement and x = the diffractometric reading, quite in the same way as before, and the numerical values of a and errors calculated are as follows:

$$y = ax \pm 0.05 \mu,$$

where

$$a = 1.123 \pm 0.0015$$

The linear relation between the values

obtained by the direct and diffractometric measurements is obvious. Notwithstanding that the values of blood specimens *both* with normal and pathological anisocytosis are equally taken and plotted in the

TABLE 1.

Comparison of the direct measurement and the monochromatic diffractometry with 25 normal and pathological blood samples.

No. of specimen	Direct measurement μ	Diffractometric measurement Reading
14	9.25	8.31
13	6.67	5.93
16	7.76	6.92
9	7.63	6.85
2	8.01	7.17
17	8.01	7.08
7	8.79	7.90
20	7.78	6.98
10	7.99	7.13
6	7.81	6.95
4	7.65	6.92
25	6.85	5.99
33*	8.39	7.43
50*	8.16	7.23
27*	7.67	6.82
40*	8.95	8.00
37*	7.39	6.55
48*	7.30	6.54
49*	8.88	7.95
43*	7.84	6.89
36*	6.85	6.09
41*	8.27	7.26
51*	8.14	7.41
47*	7.12	6.30
42*	8.21	7.40

* Specimen with pathological anisocytosis.

figure, the maximum error of the individual measurement is only 0.2μ or so, and it will be seen that this error is of the same order as the

Fig. 2. The relationship between the values obtained by the direct measurement and by the monochromatic diffractometry in wet condition.

Abscissa: Mean diameter in μ by direct measurement.

Ordinate: Diffractometric reading by monochromatic light.

○.....Blood without pathological anisocytosis.

●.....Blood with pathological anisocytosis.

error of the original method by Haden-Hausser's erythrocytometer only in cases of blood without pathological anisocytosis.

In the course of this experiment, I also found that the diffractometric reading with the monochromatic light did not change whether

the specimens fixed in methyl alcohol had been stained by Giemsa's solution or left unstained, and again whether they were mounted in saline or in distilled water (Table 2). Therefore, it seems to me that the measurement by means of a monochromatic light as the

TABLE 2.

The influence of the staining and of the difference in mounting medium on the reading of the monochromatic diffractometry.

No. of specimen	Mounted in saline		Mounted in distilled water	
	Unstained	Stained	Unstained	Stained
84	7.08	7.16	7.12	7.11
99	7.78	7.60	7.70	7.69

source with specimens fixed in methyl alcohol, unstained and mounted in distilled water is the simplest but the most convenient and reliable method in the diffractometry with this apparatus.

SUMMARY.

(1) The light source of Haden-Hausser's erythrocytometer was replaced with a monochromatic Natrium-lamp of the wave length of 5,890-96 Å.

(2) The value of the mean diameter of erythrocytes obtained by the direct measurement and the readings obtained by such diffractometry were compared in cases of the specimens with and without pathological anisocytosis, and the linear relation between the two values was obtained, the error of the diffractometry being in no case exceeding $0.2\ \mu$ and usually less.

(3) A simplification in preparing the blood specimen for such a monochromatic diffractometry is mentioned.

REFERENCES.

- (1) Allen, A and E. Ponder, Journ. of Physiol., 1928, **66**, 37.
 - (2) Matsuda, K., Tohoku J. Exp. Med., 1941, **40**, 321.
 - (3) Matsuda, K., Tohoku J. Exp. Med., 1941, **40**, 330.
-