PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 145, 192–195 (1974)

The Definition of Erythrocyte Osmotic Fragility in Normal and Abnormal Blood (37775)

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The measurement of osmotic fragility of erythrocytes can be an easy and rapid routine laboratory test. As shown by Danon and co-workers (1, 2), the automatically recorded osmotic fragility curve of blood or of its derivative ("fragiligrams") are quite different in normal fresh blood than in blood which has been stored for several weeks. David, Lustig and Nahas (3) also showed that the fragiligrams of blood sampled from patients with thalassemia major or with spherocytosis have a very abnormal shape.

These authors expressed their measurements by correlating the start and the completion of hemolysis to % dilution of an isotonic salt solution with distilled water: in normal blood, Danon reported that hemolysis starts at about 0.55–0.42% NaCl (165 to 126 mOsm) and is complete at 0.34—0.30% NaCl (102 to 90 mOsm). However, it was also observed that blood from patients with blood dyscrasias presents similar values for the start and the end of hemolysis (3). All of these measurements performed in normal and abnormal cell populations present a considerable standard deviation and were not significantly different. The interpretation of a "fragiligram," in order to be meaningful, requires, therefore, a more precise definition.

In the present studies, two types of "fragiligrams" were recorded: one which relates light scattering of the erythrocytes to osmolarity, the second which is a derivative of the first. These curves permitted the calculation of an "osmotic fragility index" of blood which defines numerically normal and abnormal cell populations.

Method. The present instrument, which will be described in detail in a separate publication, consists of light emitting diodes which transilluminate a sample of blood contained in a 15 ml test tube. Only carefully matched test tubes are used. The blood is diluted

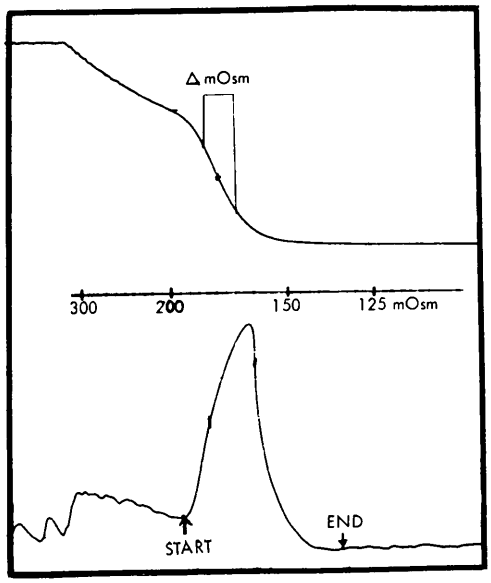

FIG. 1. Fragiligrams of normal blood. The upper curve relates light scattering of the erythrocytes (hemolysis) to osmolarity of the suspension medium. The lower curve is a derivative of the upper one, and is a display of the erythrocytes undergoing hemolysis as osmolarity of the solution is decreased. Using these two curves, it is possible to define the change in osmolarity (△ mOsm) during which hemolysis proceeds at the highest rate in 50% of the cell population (from 25 to 75%). *The curvilinear excursion of the recorded pen accounts for the curvilinear shape of the lower curve.* "Start" and "end" correspond to the start and end of hemolysis.

TABLE I. Osmotic Fragility Index of Blood from Normal Subjects and Patients with Blood Dyscrasias.

Diagnosis	Start hemolysis (mOsm)	Maximal rate of hemolysis (mOsm)	End hemolysis (mOsm)	Δ mOsm
Normal	192 ± 5.7	161 ± 3.3	128 ± 4.6	
				11.8 ± 1.12
n = 10	(180–198)[a]	(155–164)	(124–136)	
Hodgkin's	187 ± 7.4	163 ± 7.2	122 ± 5.7	
				23.4 ± 5.69
n = 18	(174–202)	(145–171)	(96–146)	
Lymphosarcoma	182 ± 5.3	155 ± 7.9	130 ± 9.4	
				18.6 ± 1.79
n = 6	(171–189)	(146–167)	(118–143)	
Thalassemia minor	205 ± 7.4	167 ± 4.9	133 ± 5.6	
				27.4 ± 6.10
n = 6	(192–214)	(158–174)	(121–146)	
Polycythemia vera	203 ± 5.3	169 ± 5.9	127 ± 5.4	
				27.1 ± 3.52
n = 5	(195–217)	(164–186)	(115–136)	
Hereditary spherocytosis[b]	204.6	182.9	176.7	31
n = 1				
Lymphoma	198.4 ± 13.9	164.3 ± 6.8	136.4 ± 3.7	
				17.1 ± 2.6
n = 4	(176.7–217)	(155–173.6)	(130.2–139.5)	

[a]Number in parenthesis indicate range.

[a]Both the daughter and the son of this subject show spherocytosis trait and had OFI = 24.8 and 18.6, respectively.

(1/600) in isotonic saline. A roller pump delivers distilled water to the sample at a constant rate of 0.33 ml/min so as to progressively decrease osmolarity and produce hemolysis. Before starting the test, the instrument is calibrated by adjusting an iris diaphragm located between the light source and the test tube, and the gain is adjusted on the recorder accordingly. This calibration serves the purpose of standardizing each sample to take into consideration individual differences in red cell volume and a wide range in hematocrit (30—60%). Two curves are recorded; one which relates light scattering by the erythrocytes during hemolysis to osmolarity of the suspension medium, the second which is a derivative of the first, *i.e.*, the rate of change of light scattering as a function of the change in osmolarity. This derivative is a display of the distribution curve of erythrocytes undergoing hemolysis. Using these two curves it was possible to define an osmotic fragility index for a given erythrocyte population; this index was defined as the range in osmolarity (Δ mOsm) within which the erythrocyte population of normal healthy donors decreases from 75 to 25% of its original value. This range was selected so as to bracket the peak of the derivative of the fragiligram and so as to define the change in osmo-

TABLE II. Osmotic Fragility Index of Blood Samples from Patients with Sickle Cell Disease.[b]

Diagnosis	No.	ΔmOsm
SS[a]	6	25.8 ± 3.01
SA	4	40.4 ± 0.4
SC	1	21.7
SC and F + A	1	40.3
S Thal.	1	21.7
S−	1	34.3
Total	14	30.2 ± 7.

[a]Includes one SS + A (after transfusion) and one SS and F.

[b]Mean value for two patients, including one with iron deficiency also; the other two had a fragiligram too flat to determine a precise numerical value.

larity at which the rate of hemolysis of normal erythrocytes is maximum (Fig. 1).

This osmotic fragility index was calculated in blood samples from normal man and from patients with blood dyscrasias.

Results. In normal men and women, the blood osmotic fragility index was 11.8 ± 1.1 mOsm (Table I). Osmolarity was calculated on the basis of 310 mOsm = 0.9% saline solution at 25°. In a number of patients with blood dyscrasias this index was significantly different (Tables I and II). It is evident that this measurement of osmotic fragility is the only one which is significantly different in normal and abnormal cell populations. The values for osmolarity corresponding to the start, the end or the maximum rate of hemolysis present a considerable standard deviation and markedly overlap. One of the original findings of this study was that blood sampled from patients with Hodgkin's disease or with lymphosarcoma presented an abnormal osmotic fragility index. Such abnormality, though not unsuspected, had never been clearly documented.

Discussion. The osmotic fragility index (OFI) which we have defined is a sensitive, precise measurement which, in normal blood, varies within narrow limits, as do all well-regulated major physiological variables. The fact that it had not yet been defined was due to the lack of a proper automated instrumentation to display a fragiligram indicative of the rate of hemolysis in function of osmolarity change.

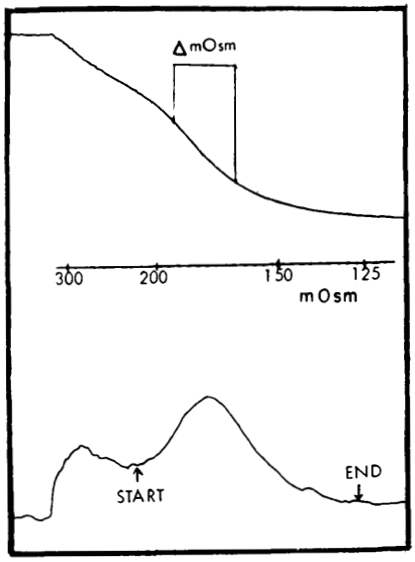

FIG. 2. Fragiligrams of blood from a patient with thalassemia minor.

This measurement can be rapidly per-

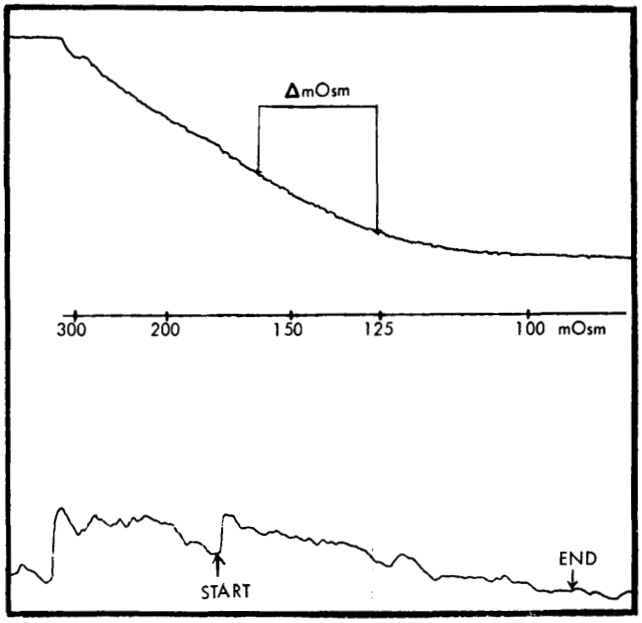

FIG. 3. Fragiligrams of blood from a patient with sickle cell trait (SA).

formed in a drop of blood and it allows for rapid screening of patients with blood dyscrasias. Such a measurement is not specific for each blood dyscrasia; but when it is abnormal, further tests are required to determine the exact diagnosis. Since this osmotic fragility index is significantly abnormal in patients with a heterozygote trait of sickle cell (SA) and with thalassemia minor, it could be a useful screening procedure to diagnose these conditions (Figs. 2 and 3).

All the patients with Hodgkin's who presented an abnormal osmotic fragility index were in different active stages of the disease, and half of them had not received radiotheraphy. A shortened life span of erythrocytes from patients with Hodgkin's disease was previously reported (4). The mechanism of this condition and its presence or absence during the remission of the disease will be interesting to investigate.

Patients with lymphosarcomas and lymphomas also presented an abnormal osmotic fragility index. This condition is an indication of the anemias associated with cancer (5).

Summary. The osmotic fragility of blood can be numerically defined by measuring the change in osmolarity (Δ mOsm) required to hemolyze 50% of the erythrocyte population when hemolysis is proceeding at the highest rate. In normal blood Δ mOsm = 11.8 ± 1.1 mOsm. In all blood dyscrasias which were studied this osmotic fragility index was significantly different from normal. It was abnormal in the bearers of sickle cell or thalassemia trait. It was also abnormal in Hodgkin's disease and in cancer patients.

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Received July 18, 1973. P.S.E.B.M., 1974, Vol. 145.