Kawasaki Med. J. Vol. 2: No. 2, 99-105, 1976

PROPERTIES OF ERYTHROCYTE MEMBRANE IN A HYPOTONIC MILIEU OF DECREASING OSMOLARITY (COIL PLANET CENTRIFUGATION)

Osamu YAMADA

Division of Hematology
Department of Medicine, Kawasaki Medical School,
Kurashiki, 701-01, Japan

Accepted for Publication on Feb. 10, 1976

Abstract

A column of aqueous sodium chloride solution having a rectilinear osmotic gradient (150→30mOsM) was set on a coil planet centrifuge (CPC), which was filled with 10μl blood (which was inhibited from coagulation with Anticlot Et, and was used just after the treatment or after allowing to stand at room temperature for 4-6 hours); both ends of the column were sealed by heat-fusing, and after heating at 37°C for 10 minutes, it was centrifuged by CPC (37°C for 10 minutes) to observe the membrane properties of erythrocytes in a dynamic state with continuous decreasing of the osmotic pressure. One hundred normal subjects (49 males and 51 females) were examnied by this method and the following values and hemolysis band were obtained.

Hemolysis starting point (HSP): 98.7±SD 5 mOsM
Hemolysis end point (HEP): 63.8±SD 4 mOsM
Hemolysis band shape: L type (sometimes M type)

Thus, there may be employed HSP 100 mOsM and HEP 65 mOsM as the approximate standard values, and L type may be considered to be a standard from of the hemolysis band.

Seasonal variation was seldom observed, and was slight, if any.

INTRODUCTION

For examining the membrane properties, usually erythrocytes are put in a series of test tubes of hypotonic saline to observe occurrence of hemolysis and its degree: the typical procedure is Parpart's erythrocyte osmotic fragility test[1] which has been widely used for diagnosis of hereditary spherocytosis.

In Japan coil planet centrifugation (CPC) has been tried for these several years, in which erythrocytes are driven from a high osmolarity side to a low osmolarity side in a column of solution having rectilinear osmotic gradient, and exposed to the continuously accelerated hypo-

osmolar stress to observe the membrane properties of erythrocytes quantitatively based on the hemolysis band found there[2]. By this method erythrocytes are continuously driven toward the lower osmotic solution to observe the strength of the membrane under a "dynamic condition", contrary to Parpart's method and conventional procedures in which erythrocytes are put in a solution of static hypo-osmolarity to observe hemolysis under the so-called "static condition".

CPC is applied to the test for various hematologic diseases including hemolytic anemia and evaluated as a method which can give some observations that cannot be obtained by the erythrocyte osmotic fragility test in the "static state"[2]: moreover, since Kitazima and Shibata (1975)[3,4] tried to use it on patients of hepatobiliary disorders and reported it was useful for observing the clinical course and estimating prognoses of these diseases, it aroused general interest and come to be used widely[5-9].

It must be noted that CPC has been improved recently to accomplish its mechanism, and that, accordingly, it is indispensable to standardize the procedure for testing the membrane properties of erythrocytes and to determine the standard values of a normal person by testing his erythrocytes. The author tested 100 normal subjects by the standardized CPC to observe the membrane properties of erythrocytes to determine the normal values (hemolysis starting and end points and hemolysis band shape). The results obtained are reported here, and also some matters relevant to this method are discussed (seasonal variation, the time from blood collection to the test, etc.).

METHOD

1) Materials: For determining normal values, 1 ml blood each was collected from the antecubital veins of 100 normals adults (49 males and 51 females) with a disposable shringe; to the collected blood a drop of Anticlot Et was added and blended to inhibit coagulation, and after allowing to stand at room temperature for 4-6 hours, it was tested.

For observing seasonal variation, the blood was collected similarly from three of the same normal adults (2 males and 1 female) once a month to serve as specimens. The blood was also collected from 181 normal adults including those who came to Blood Transfusion Center for donation and others.

2) Coil planet centrifugation[4]: A nozzle of a gradientor (which was provided with springe barrels A and B filled with NaCl aqueous

solution of 150 mOsM and 30 mOsM respectively and could supply a NaCl solution having a high → low rectilinear osmotic gradient as changing the blending ratio of the solutions of A and B automatically) to a coil (which was prepared by winding an acrylite rod of 20 cm long and 5 mm thick with polyethylene tube of 3 m long and 0.3 mm inner diameter, and was corresponding to the centrifuge tube of a conventional centrifuge); thus, 150 → 30 mOsM solution column was prepared, and it was allowed to aspirate 10 μl blood specimen (corresponding to 6 cm deep in the polyethylene tube of the coil) from the high osmolarity side; the both ends of the coil tube were pinched by heated iron to seal by fusion, and caps were put on the both ends of the acrylite rod of the coil.

The coil containing the blood was put in a warming box (37°C) for 10 minutes to heat up, and then put in a coil-holder groove of CPC with the high osmolarity side down; it was covered with a case, fixed with a clasp, and after drawing a cover down, a switch provided with a timer was put on to begin to centrifuge at the inner temperature of 37°C for 10 minutes.

When the time was up, the coil was taken out of CPC, put on a graph sheet for recording the coil position-osmolarity (Fig. 1) to read the osmolarity of the both ends of the hemolytic band (the higher osmolarity side……the hemolysis starting point: the lower osmolarity side……

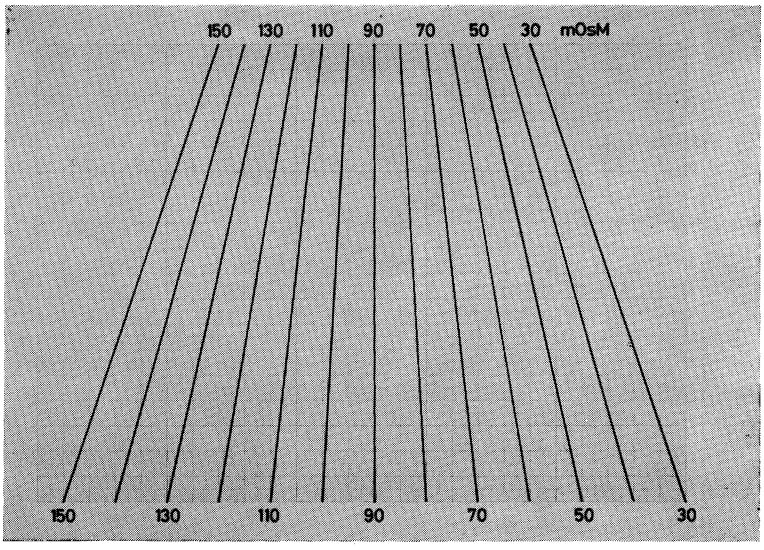

Fig. 1. Graph sheet for reading hemolysis "starting" and "end" points of a hemolysis band in terms of osmolarity.

the hemolysis end point), and then sent to the densitometer for observing the photoelectric colorimetry of the degree of hemolysis (the strength of the scarlet hue) at each position of the hemolysis band to record automatically the densitograph on the recording paper as a curve.

RESULTS

The normal values obtained are summarized in the following (1) to (3).

(1) The hemolysis starting point (HSP)[4] and the hemolysis end point (HEP)[4] of the hemolysis band:

Normal person (100 subjects): HSP 98.7±SD 5
HEP 63.8±SD 4
Male (49 subjects): HSP 98.6±SD 5, HEP 62.7±SD 4
Female (51 subjects): HSP 98.9±SD 6, HEP 64.9±SD 4

(2) When observing the shape of the hemolysis band by densitography, as seen typically in Fig. 2, there were found L type (whose left

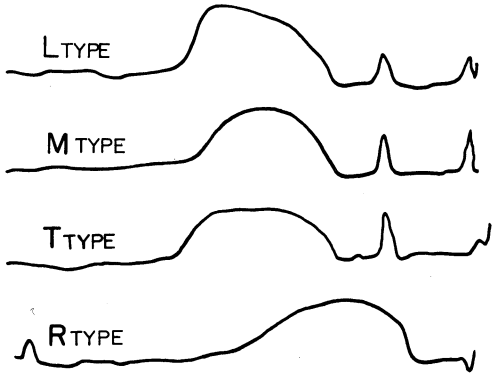

Fig. 2. Classification of the patterns hemolysis bands in the "coil" as observed by photoelctric densitography. L type: The hemolysis curve with its peak shifted to the higher osmolarity end (Left side), M type: The hemolysis curve with its peak in the middle, T type: The hemolysis curve with a broad table-like peak in the middle portion, R type: The hemolysis curve with its peak shifted to the lower osmolarity end (right side).

shoulder raised……the peak of hemolysis was near the high osmolarity end), M type (the peak of hemolysis was near the center, and appeared to be like a normal distribution curve), T type (the peak of hemolysis was at the center, but it extended to both sides to be like a plateau or a table), and R type (the peak of hemolysis was shifted to the right side, i.e.,

the low osmolarity side, and appeared to have a raised right shoulder). The frequencies of these types were:

Type	L	M	T	R
Frequency (%)	75	15	5	5

(3) Seasonal variation: Fig. 3 shows HSP and HEP of normal adults (including donors coming to the blood transfusion department); seasonal variation was not clear. There was seen a tendency that HSP and HEP slightly shifted to the low osmolarity side in summer (July and August) and in fall and spring they returned to the original positions.

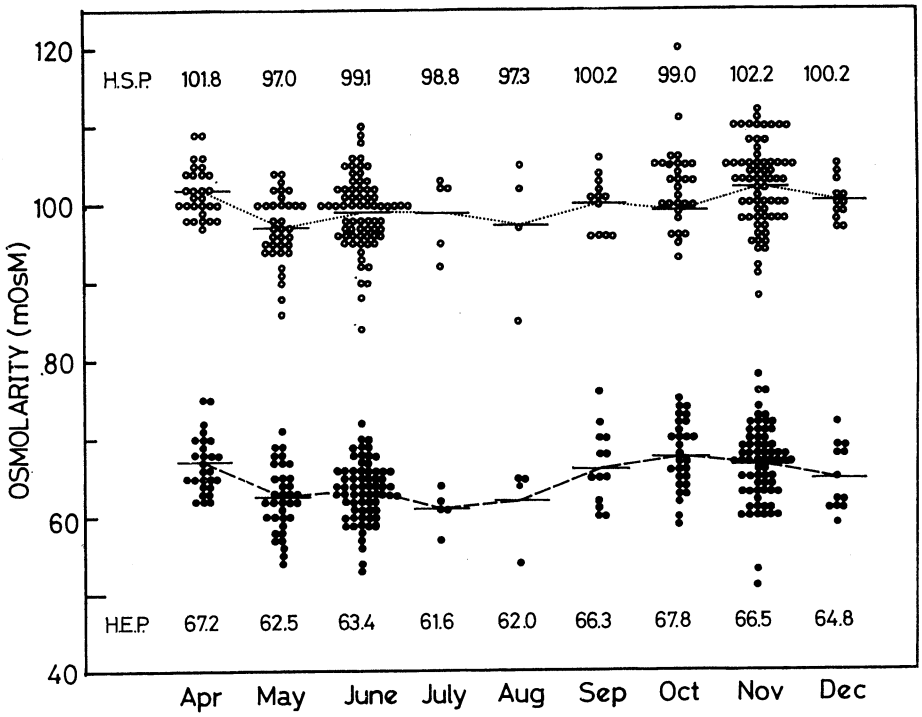

Fig. 3. Seasonal variation of the hemolysis "starting" point (HSP) and the hemolysis "end" point (HEP) in normal subjects.

DISCUSSION

According to the results described above, erythrocytes of a normal person yielded a hemolysis band in which HSP and HEP were distributed in the ranges of 99 mOsM and 64 mOsM with ±SD4-5 mOsM, respectively, when tested by CPC with the coil filled with NaCl aqueous solution

having the osmolarity gradient of 150 → 30 mOsM. The normal range of the hemolysis band hardly showed the difference by sex. However, the blood almost 2 hours after collecting gave HSP and HEP shifted to the high osmolarity side by about 5 mOsM as compared with the blood just after collecting (and after allowing to stand for 4-6 hours at room temperature). When the atmospheric temperature was low (for example, 0°C or the temperature in an ice box), HSP and HEP of the blood of a normal person showed the identical values as those observed just after collection of the blood, even when the blood was allowed to stand for 6-48 hours. However, when the temperature was over 30°C, the both ends of the hemolysis band (HSP and HEP) shifted to the high osmolarity side in many cases.

When the collected blood was put in a test tube which was then stoppered airtight and kept in a thermostatic water bath (37°C) for 24 hours, HSP and HEP shifted to the high osmolarity side by 30 mOsM and 4 mOsM on an average, respectively, as compared with the values obtained just after collection. It is interesting and noteworthy that when the blood of a patient of hepatobiliary disorders was heated at 37°C for 24 hours similarly as described above, the hemolysis band shifted to the low osmolarity side, and that particularly it was obvious on HEP.

The shape of the hemolysis band (densitograph) should be considered normal when it is L type, and if not, it is M type. T type and R type suggest a possibility of anomaly. In fact, the author has frequently experienced that the shape which was R type in the active stage of hepatitis changed to M type → T type → L type in the course of convalescence.

As described in the introduction of this report, observation of the membrane properties of erythrocytes by CPC has been tried for diagnosis of diseases for these several years in Japan[2-10]. For understanding the findings correctly, it is necessary to keep the conditions of observation constant and to establish normal values and normal findings. The experiment in the present report was performed for this purpose.

REFERENCES

1) Dacie, J. V. and Lewis, S. M.: Practical Haematology 4th ed. Churchill (London), 1968
2) Shibata, S., Kobayashi, K. and Sera, Y.: Application of coil planet centrifugation to hematology—Measurement of osmotic fragility of erythrocytes and autohemolysis test J. Japan Med. Instrum 40: 619-626, 1970
3) Kitazima, K.: Measurement of erythrocyte osmotic fragility in hepatobiliary disorders by coil planet centrifugation. Igaku to Seibutsugaku, 87: 119-124, 1973

4) Kitazima, K. and Shibata, S.: Coil planet centrifugation and its application to the observation of altered membrane properties of erythrocytes in hepatobiliary disorders J. Lab. & Clin Med. 85: 865-864, 1975
5) Yamashita, Y., Kitazima, K. and Shibata, S.: Measurement of erythrocyte osmotic fragility by coil planet centrifugation. Jpn. J. Clin. Pathol., 21 (Supplement): 154-154, 1973
6) Fukuoka, K., Maeda, K. and Kubo, M.: A new trial about measurement of erythrocyte osmotic resistance by coil planet centrifugation (2nd report). Jpn. J. Clin. Pathol., 21 (Supplement): 155-155, 1973
7) Maeda, H., Kubo, M. and Fukuoka, K.: A new method for osmotic fragility test of erythrocytes with coil planet centrifuge. Jpn. J. Clin. Hematol., 15: 501-507, 1974
8) Kawagoe, S. and Enami, T.: On the difference of hemolysis of aged and young erythrocytes and the pattern of hemolysis bands in various anemic diseases as observed by coil planet centrifugation. Jpn. J. Clin. Pathol., 22 (Supplement): 123-123, 1974
9) Kawagoe, S.: Diagnosis of hematological diseases by coil planet centrifugation with special reference to aggregation-hemolysis pictures. Jpn. J. Clin. Pathol., 23 (Supplement) 527-527, 1975
10) Takagi, K. and Kawai, T.: Studies on osmotic resistance of antibody-sensitized erythrocytes and their morphological changes. Jpn. J. Clin. Pathol., 23 (Supplement): 527-527, 1975