RBC Osmotic Fragility Test Using Immunological Microplate

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Introduction

There are some methods concerning laboratory work for the diagnosis of hemolytic diseases : measurement of osmotic fragility, measurement of mechanical fragility, autohemolysis test and other more specific tests for some special disease conditions. The osmotic fragility test of RBC, first proposed by Hamburger (1883), has been modified many times. Methods proposed by investigators are summerized in Table 1. Those methods can be divided into three groups : visual method, photometric method, and automatic recording method. All the methods examine presence of free hemoglobin in the hypotonic NaCl solution in which RBC are suspended. Type of blood sample used in the test is either whole blood or defibrinated blood or anticoagulated (citrated or oxalated) blood and the amount needed is at least several ml. There have been few methods of measuring osmotic fragility of RBC in which hemolysis is evaluated by the decrease of RBC amount (or number).

In this paper, the author report a new micro-method requiring only 0.1 ml blood. The blood sample can be obtained from either venous blood or capillary blood

Methods and Materials

I. Method of osmotic fragility of RBC

Following method is adapted as a control of the new micromethod which will be mentioned later.

Main Equipment:

1) Calibrated serological pipette, 5 ml., 1 ml., and 0.5 ml.,
2) International Centrifiuge, size 2.
3) Bausch and Lomb Spectrophotometer.

Reagents:

NaCl stock solutions with following serial concentrations:

1.00 % 0.95 % 0.90 % 0.85 % 0.80 % 0.75 % 0.70 % 0.65 %
0.60 % 0.56 % 0.52 % 0.48 % 0.44 % 0.40 % 0.36 % 0.32 %
0.28 % 0.24 % 0.20 %

Blood Sample:

Oxalated venous blood.

Procedure:

1) Prepare 20 kahn tubes of serial NaCl solution & H_2O, mark on the tube wall and put on the rack in serial order. With a 1 ml. serological pipette transfer 1. ml. dist. water to the tube H_2O, using the same pipette, transfer 1 ml. of 0.20 % NaCl solution to the tube 0.20; 1 ml. of the serial NaCl Solutions is

transferred to the corresponding tube in the same manner.

2) Using a 1 ml. serological pipette add exactly 0.1cc of the well mixed oxalated blood to each tube, place a rubber cap on each, and invert several times to obtain thorough mixing.

3) Allow to stand for 20 minutes at room temperature, then centrifuge at 2,000 r.p.m. for 10 minutes.

4) Visual determination of initial and complete hemolysis:
After the tubes are taken out from the centrifuge, initial hemolysis & complete hemolysis are read through the naked eye. The highest NaCl concentration in which supernatant is tinged with pink color, and the highest NaCl concentration in which no RBC package can be seen in the sediment are regarded as initial hemolysis & complete hemolysis, respectively.

5) Prepare 20 photometer cuvettes to which serial mark is drawn on the tube wall and 4.5 ml. distilled water is placed in each tube. With a 0.5 ml. serological pipette transfer 0.5 ml. supernatant of tube 1.0 to photometer cuvette, the inner wall of the pipette, is washed by sucking & blowing out the distilled water several times. The same procedure is performed in the order from high to low NaCl concentration.

6) Invert each cuvette several times to mix homogenously.

7) The % of transmission rate is read on B & L spectrophotometer with the wavelength 540 mμ then the optical density (O.D.) is calculated. The supernatant solution from tube 1.0 is used as a blank. The tube containing H_2O is taken to be 100 % hemolysis and the percentage hemolysis in each of the other tubes is calculated by dividing the O.D. by the O.D. of the tube H_2O and multiplied by 100.

8) The % of hemolysis in the serial hypotonic NaCl solution is plotted on the graph paper & a curve is drawn, as seen in Fig. 1.

II. A new micromethod using microtiter plate

1. Principle of the method

The hemolytic curve described by Dacie, Parpart, etc. shows the relative amount of RBC destroyed in the serial hypotonic NaCl solution (Fig. 1). If one draws the curve with a thick line one can express not only the relative amount of RBC hemolysed but also the relative amount of RBC (number per packed volume) which remains safely in the hypotonic solution. (Fig. 2) While all the methods of examining osmotic fragility of RBC stress the decrease of free hemoglobin in NaCl solution, there may be a method in which, to know the hemolysis, charge of packed RBC volume is observed. It is thought the latter method seems to be less sensitive than the former method. It is needless to say, however, that a more sensitive method is always superior to a less sensitive one. When a limited volume of RBC suspension is serially diluted and the suspension series are centrifuged, the size of visible packed RBC may be found to become smaller & smaller as the dilution increases and finally gross packed RBC (red color spot) becomes invisible though RBC may still be detected microscopically.

Points of minimal hemolysis (or initial hemolysis) and maximal hemolysis (or complete hemolysis) can be detected when a certain concentration of RBC suspension is used in the osmotic fragility test. That is, to detect the former point, every highly diluted RBC suspension is used. For example, the size of packed RBC of 1/10,000ml. of the

RBC suspension can be visible through the naked eye when it is centrifuged in a special container but is so small that if some small part of RBC is removed as the result of hemolysis the packed cell volume becomes invisible The percentage of NaCl of the tube shows the smallest RBC dot which is followed by the tube with invisible RBC dot can roughly be regarded as minimal hemolysis (Fig. 4)

In case of detecting maximal hemolysis the principle is just the same provided that the concentration of RBC suspension must be higher. The point of maximal hemolysis may be % of NaCl of the first tube in which no more RBC package can be visible, (Fig. 3).

The sensitivity of the change of RBC package in hypotonic NaCl dilution series may be variable but it may be sensitive to 5% - 1.0% hemolysis if 0.1 cc of 1/200 & 1/1,000 RBC suspension is used. It will be discussed in detail later.

2. Preliminary experiment

(1) Determination of an adequate RBC amount (or number) to examine "initial hemolysis" or "minimal hemolysis" point.

Normal oxalated blood which is known to have about 45% of hematocrit value is diluted with 0.9% NaCl solution to make various concentrations of RBC suspension, such as 1/100 1/200, 1/400, 1/800, 1/1000,……, etc. (as seen in Table 2). 0.1 ml. of each RBC suspension is placed in the holes of microtiter plate with sharp-pointed bottom (Fig 5). It is centrifuged at 2,000 r. p. m. for 10 minutes, and then, the size of RBC dot seen at the bottom is compared with each other. 0.1 ml of 1/5,000 dilution of normal blood is the lowest that the RBC dot can be seen definitely through the naked eye (Fig. 6-1).

(2) Determination of an adequate concentration of RBC suspension to determine "complete hemolysis" or "maximal hemolysis" point

Reagent: 14 stock solution of NaCl with decreasing strength (0.90% 0.44% 0.40% 0.36% 0.32% 0.28% 0.24% 0.20% 0.16% 0.12% 0.10% 0.08% 0.05% H_2O).

Procedure:

1) Normal oxalated blood which is known to have about 45% of hematocrit value is diluted with 0.9% NaCl solution to make varying concetrations of RBC suspensions such as 1/10 1/20, 1/30, 1/40, 1/50, 1/100, 1/200; as seen in Table 3-1.

2) Seven lines of 14 Kahn tubes each of which is marked are prepard. Using a 0.5 ml serological pipette 0.4 ml H_2O is placed in tube 4 then using the same pipette, 0.4 ml of NaCl solution series is placed in such an order that the pipetting is done from low to high concentration.

3) With an/0.5 ml serological pipette 0.1 ml of RBC suspension is added to 14 Kahn tubes. RBC suspension should be thoroughly mixed while pipetting is done. The procedure is repeated for all seven different concentrations of RBC suspension (as seen in Table 3-2).

4) After thorough mixing with a 0.5 ml serological pipette transfer 0.1 ml RBC suspension from each Kahn tube to the hole of microtiter plate. For each RBC concentration using the same serological pipette, tube 14 is transferred first, then tube 3 and so forth.

5) Centrifuge the microtiter plate at 2,000 r. p. m. for 10 min.

6) Reading of the result:

The size of the packed RBC dot in the bottom of the hole is observed & recorded as 5+ to —(Table 3-3). The relative size of the packed RBC dot at the bottom of the hole is drawa (Fig. 6-2).

(3) Sensitivity of RBC suspensions to hemolysis

Mix 0.1 ml normal oxalated blood which is know to have about 45% of hematocrit value with 19.9 ml of 0.9% NaCl solution to make 1/200 RBC suspension and mix 3 ml of 1/200 RBC suspension with 12 ml 0.9% NaCl solution to make 1/1,000 RBC suspension.

Two lines of 21 Kahn tubes each of which is marked are prepared In tube No.1 place 1.0 ml of 1/200 RBC suspension (Or 1/1,000 RBC ··· suspension) ; in tube No.2 place 0.95 ml of 1/200 RBC suspension (or 1/1,000 RBC suspension) and 0.05ml of 0.9%NaCl solution The procedure is tabulated in Table 4-1 & Table 4-2. In each Kahn tube the total volume of suspension is 1.0 ml, The relative amount of RBC in tube No. 1 is 100, the relative amount of RBC in tube No. 2 is 95, and so forth.

After thorough mixing, with a 0.5 ml. serological pipette transfer 0.1 ml of RBC suspension from each Kahn tube to the hole of microtiter plate. For each concentration of RBC suspension tube No. 21 is transferred first, then tube No. 20, and so forth. Centrifuge the microtiter plate at 2,000 r.p.m. for 10 minutes. The result is shown in Tables 4-1 & 4-2 and Fig 7-1 & Fig 7-2

As seen in Fig. 7-1 & Fig. 7-2 destruction of only 5% RBC in 1/2,000ml. and 1/10,000 ml of the blood can be clearly differentiated.

(4) Conclusion

From the results shown on Table 2 & Fig. 6-1, the packed RBC dot of 1/20,000 ml 1/30,000ml, 1/40,000 ml, or 1/50,000 ml normal blood can be seen through the naked eye, But these four RBC dots are all so small that any technical error shch as inadequate mixing during pipetting the RBC suspension may render the size of RBC dot invisible. The amount of normal blood used in preliminery experiment (2) is larger than 1/15,000 ml.

From the results shown on Table 3-3 & Fig. 6-2 the initial decrease in size packed RBC dots can be seen through the naked eyes both in 1/5,000 & 1/10,000 ml normal blood at 0.404% & 0.436% NaCl solution respectively. According to the principle of the new micro-method as already mentioned to determine the point of minimal hemolysis will be more sensitive to use higher diluted permissible concentration of RBC suspension. So 1/10,000 ml of normal blood is adopted to determine the point of minimal or initial hemolysis.

Complete disappearance of RBC dot in each suspension (1/500,1/1,000,1/1,500,1/2,000 & 1/2,500 ml) of normal blood occurs at tube No. 8, that is 0.34% NaCl solution as shown in Table 3-3 & Fig. 6-2. Among them stepwise decrease of the size of RBC dot is more clearly seen in the series of 1/2,000 & 1/2,500 ml of normal blood. It will be suitable to use 1/2,000 or 1/2,500 ml of normal blood in the determination of maximal or complete hemolysis point.

3. A new micromethod using microtiter plate

Equipment:

1) Kahn Tubes
2) Microtiter plate
3) Calibrated serological pipette, 0.5ml & 1 ml.
4) International Portable Refrigerated Centrifuge Model PR-2 & adapter for centrifuging the microtiter plate.

Reagents :

1) Normal saline (0.90 %)

2) Hypotonic NaCl solution series :

(0.44 % 0.40 % 0.36 % 0.32 % 0.28 % 0.24 % 0.20 % 0.16 % 0.12 %
0.10 % 0.08 % 0.05 % H_2O)

Blood Sample :

Blood samples (oxalated venous blood) are collected from 10 healthy persons. 1/40 & 1/200 dilutions of RBC suspensions are prepared with the following formula :

normal blood	0.1 ml	0.4 ml
0.9 % NaCl	3.9 ml	1.6 ml
dilution of RBC suspension	1/40	1/200

Procedure :

1) A pair of 14 Kahn tubes is prepared. Mark number (1-14) on each tube.

2) Using a 1 ml serological pipette, 0.4 ml of H_2O is placed in each of the two tubes No. 14, then using the same pipette, 0.4 ml of NaCl solution series is placed in such an order that the pipetting is done from low to high concentration to the tubes from 13 to 1.

3) With a 0.5 ml serological pipette, 0.1 ml of 1/40 or 1/200 RBC suspension is added to each tube. RBC suspension should be thoroughly mixed while pipetting is done(refer Table 3-2)

4) After thorough mixing with a 0.5 ml serological pipette 0.1 ml RBC suspension is transferred from each Kahn tube to the hole of microtiter plate. Using the same serological pipette transfer is started from tube No. 14 to tube No. 1.

5) Centrifuge the microtiter plate at 2,000 r.p.m. for 10 minutes.

6) Reading of the result :

The size of the packed RBC dot at the bottom of the hole is observed and recorded as 4 + to - (Table 5)

Results

Osmotic fragility of RBC measured by the author's method and a modification of Parpart's method on 10 normal persons is shown in Tbble 5 and the curve are shown in Fig. 8-Fig. 18.

The determination of "minimal" and "maximal" hemolysis :

Minimal and maximal hemolysis are arbitrarily defindd as 5 % and 90 % hemolysis, respectively. The reason for the determination is to remove the abnormally fragile and unusually resistant RBC which are estimated from the fragiligraph.

Correlation between the value of macromethod and micromethod is studied for seeking minimal & maximal hemolysis point. As seen in Tables 6-1 & 6-2, it can be said that, to get the point of 5 % hemolysis, one should take NaCl % of the 2nd tube from the tube showing the smallest RBC dot at the bottom after centrifugation and, to get 90 % hemolysis point, one should take NaCl % of the 2nd tube with no visible RBC dot, to read the 10 % of % hemolysis NaCl % at the tube showing the smallest RBC dot may be taken and to read 95 % of hemolysis NaCl % at 3 tubes after the initial tube which show no RBC dot.

When the minimal and maximal hemolysis value produced by the author's micromethod and control method (both visual determination and photoelectric determination) are com-

pared. (Table 6-3) The value is similar but the micromethod seems to be rather more sensitive in the determination of minimal hemolysis and almost equal or a little less sensitive in the determination of maximal hemolysis.

Discussion

Factors which may influence the result of the osmotic fragility test are PH & temperature of the erythrocytic environment, and the type and amount of erythrocytes (defibrinated blood or anticoagulated blood) given and adequate mixing during pipetting the RBC suspension. pH of the erythrocytic environment can easily be adjusted at 7.4 by using phosphate buffered NaCl solution as in Parpart's method. As to the latter factors strict caution should be exercised in all the methods no matter whether it is visual or photoelectric colorimetric method, it seems to be more important when the size of sample is small in amount or highly diluted.

Expression of "minimal" (or initial) and "maximal" (or complete) hemolysis is commonly done to evaluate the fragility of RBC. The definition of the two points is rather arbitrary. It seems natural to take 1 % of hemolysis & 100 % of hemolysis for the two points, but when seeing the fragility curve (fragiligraph) as one can understand, there is anincrease of hemolysis at the concentration of initial and terminal phase and no 100 % hemolysis can be achieved until H_2O tube or even in H_2O tube in many cases. Between the areas of 5-10 % inemolysis & 90 % hemolysis the hemolytic curve tends to show a straight line. These phenomena mean some of erythrocytes are unusually fragile or resistant while the majority of RBC simply show gradual increase of destruction in the serial hypotonic NaCl solution.

From the author's limited experience it is rather practical that points of initial and maximal hemolysis may be taken at 5 % and 90 % of hemolysis. These two points as indicated in Tables 6-1 & 6-2 obtained by the author's new method correspond to 5 % and 90 % hemolysis respectively when compared with a modified Parpart's method.The straight line connecting the two points shows the fragility of the majority of RBC which can be ordered according to their age (Fig. 8-Fig. 18).

Based on the present author's new micromethod the following simplification of the technic can be attempted:

I. Equipment:

1) RBC & WBC pipettes
2) Microtiter plate and dropper (0.025 ml)
3) International Portable Refrigerated Centrifuge Model PR-2 & adapter for centrifuging the microtiter plate.
4) Microautomixer
5) Microtiter plate mixer

II. Technique:

A. Determination of maximal hemolysis:

1. Collect blood with WBC diluting pipette until mark 1.0 and suck 0.9 % NaCl solution until mark 11.
2. After thorough mixing & discarding the initial 3 drops, there remains RBC suspension dropped into small test tube (number of drops is counted exactly). With the same pipet 4 volumes of 0.9 % NaCl solution is added and mixed. The dilution of RBC suspension is now 1 : 50.

3. With microtiter dropper (0.025 ml) 0.025 ml of RBC suspension is placed in each hole of microtiter plate (14 holes are used).

4. Using the same dropper 0.075 ml of serial NaCl solution is added to each hole in the order from low to high concentration.

5. Microtiter plate is put on microtiter plate mixer to mix homogeneously for 3 minutes.

6. Centrifuge at 2,000 r. p. m. for 10 minutes.

7. Reading of the result.

B. Determination of minimal hemolysis :

1. Same as A-1 but RBC diluting pipet is used to prepare 1 : 100 dilution of RBC suspension.

2. Same as A-2 but addition of 0.9 % NaCl = 1.5 volumes so that one can get 1 : 250 dilution of RBC suspension.

3-6 Same as A-3 ……A-6. Techniques 3-6 can be performed on the same microtiter plate if necessary.

Summary

A new micromethod for the osmotic fragility test of RBC is introduced. Through this method determination of the minimal & maximal hemolysis points is easy and two points are known to correspond to approximately 5 % & 90 % hemolysis. It is suggested, instead of the fragility curve drawn on Parpart's method, a straight line connecting two points which is determined by this new micromethod may be used.

Acknowledgement

The author express her deep gratitude to Dr. C. H. Liu for his advice & conduction of the studies, and also to Dr. C. H. Wang for her thoughtful advice and technical help. My thanks are also due to those who have offered their opinions and/or assistance to me.

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Table 1

Method adopted by or described by	Type of Sample	Temperature	pH Adjustment	Duration of the time after mixing	Necessity of centrifugation	Reading of the result
described by Rebiere 1)	citrated blood	R.T.	—	2 hrs.	+	visual
Sanford's Method 2)	whole blood or 3 : 2 oxalated blood	R.T.	—	2 hrs.	—	visual
Sanford's Method 2) modified by Wintrobe	whole blood or 3 : 2 oxalated blood	4°C	—	2 hrs.	—	visual
described by. 3) Leavell-Thorup	heparinized blood	4°C	—	2-3 hrs.	—	visual
Dacie's Method 4)	heparinized blood	R.T.	7.4	30 min.	+	spectrophotometry
Parpart's Method 5)	citrated blood	20°C	7.4	45 min.	+	〃
described by Hunter 6)	oxalated blood	R.T.	—	30 min.	+	〃
Fennel Method 2)	fresh blood	R.T.	—	30 min.	+	〃
described by Miller 7)	heparinized or defibrinated blood	R.T.	7.4	30 min.	+	〃
described by Page & Culver 8)	oxalated blood	R.T.	7.4		+	〃
Greed's Technique 9)	oxygenated blood add heparin	R.T.	—	20 min.	+	〃
described by Levinson & Mac Fate 10)	oxygenated blood	R.T.		1 hr.	+	〃
described by Cartwright 11)	defibrinated blood	R.T.	7.4	30 min.	+	〃
Pipette method described by Cracke & Garver 12)	fresh blood	R.T.	—	1 hr.	—	counting
Danon's method 13)	heparinized blood		7.4			automatically recorded

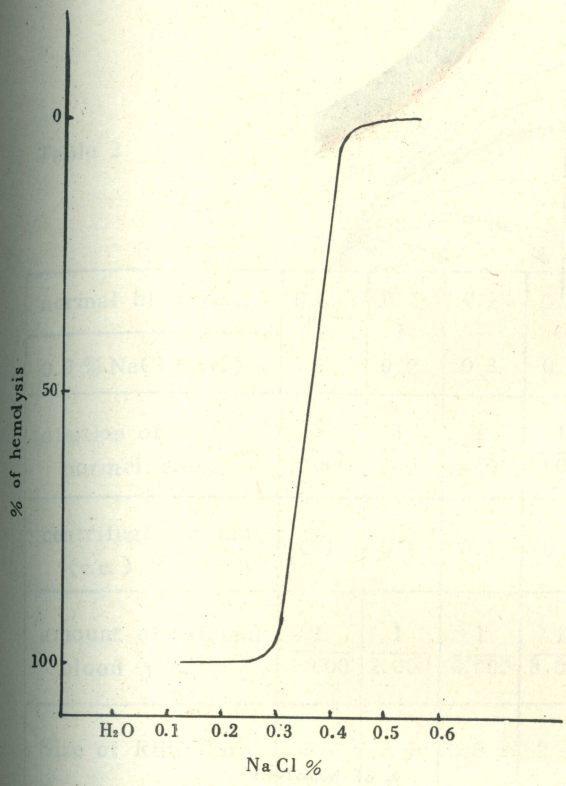


Fig. 1 Erythrocyte osmotic fragility curve described by Dacie, Parpart and others. The curve expresses the relative amount of RBC hemolysed in the hepotonic Na Cl solutions.

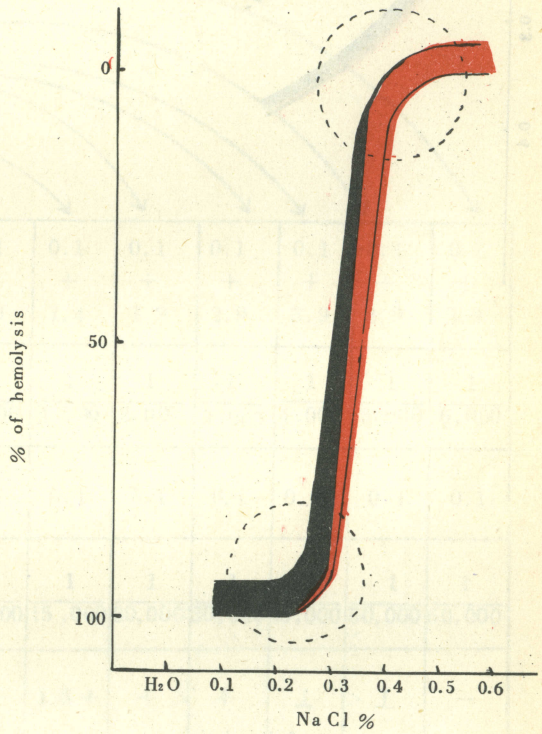
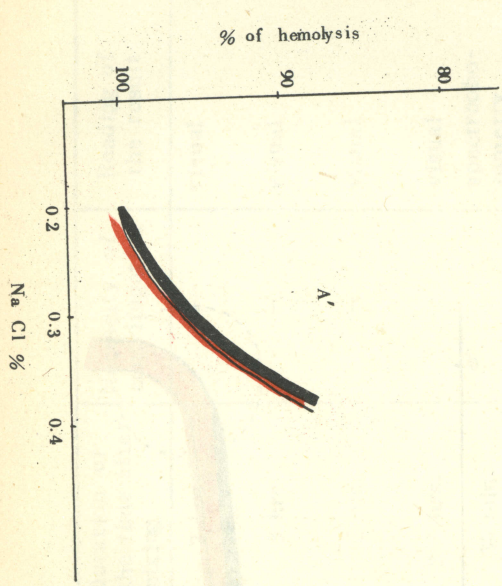
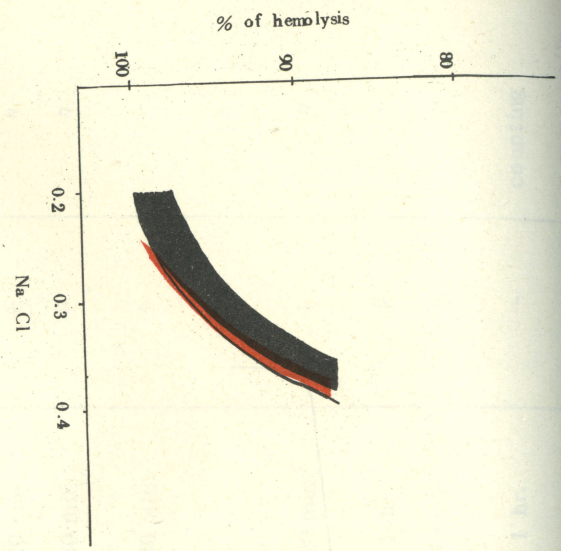
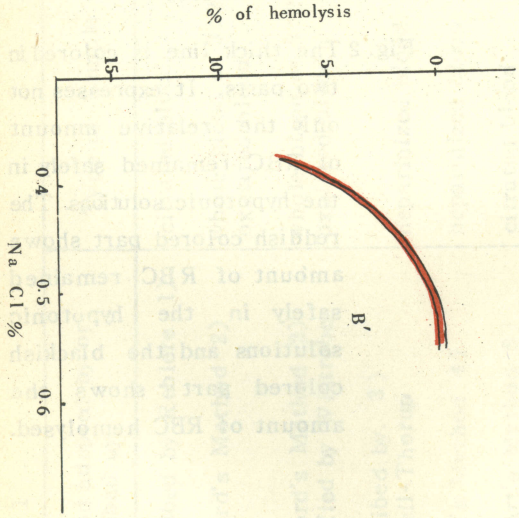
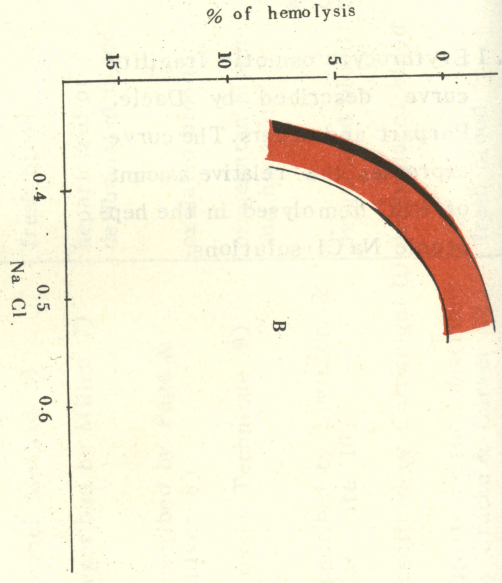


Fig. 2 The thick line is colored in two parts. It expresses not only the relative amount of RBC remained safely in the hypotonic solutions. The reddish colored part shows amount of RBC remained safely in the hypotonic solutions and the blackish colored part shows the amount of RBC hemolysed.


% of hemolysis
100
90
80
0.2
0.3
0.4
Na Cl %
A'


% of hemolysis
100
90
80
0.2
0.3
0.4
Na Cl


% of hemolysis
15
10
5
0
0.4
0.5
0.6
Na Cl %
B'


% of hemolysis
15
10
5
0
0.4
0.5
0.6
Na Cl
B

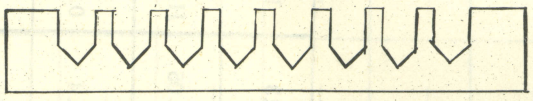


Fig. 5 Hole of microtiter plate. The diameter of the hole is 0.65 cm., the capacity is 0.2c.c and the bottom of the hole is sharp-pointed.

Table 2

normal blood (c.c.)	0.02	0.2	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
0.9 % NaCl (c.c.)	+ 2	+ 0.2	+ 0.3	+ 0.7	+ 0.9	+ 1.4	+ 1.9	+ 2.9	+ 3.9	+ 4.9	+ 5.9
dilution of normal blood	1/100	1/200	1/400	1/800	1/1,000	1/1,500	1/2,000	1/3,000	1/4,000	1/5,000	1/6,000
centrifuge amount (c.c.)	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
amount of original blood (c.c.)	1/1,000	1/2,000	1/4,000	1/8,000	1/10,000	1/15,000	1/20,000	1/30,000	1/40,000	1/50,000	1/60,000
Size of RBC dot	4 +	3 +	2.5 +	2 + >	2 +	1.5 +	+ >	+	⊥ >	⊥	−

Fig. 6-1

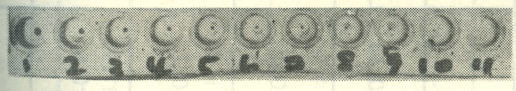


Table 3-1

normal blood (c.c.)	0.2	0.3	1.6	1.5	0.8	0.2	0.2
	+	+	+	+	+	+	+
0.9 % Na Cl (c.c.)	1.8	5.7	0.8	1.5	1.2	1.8	1.8
didution of normal blood	$\frac{1}{10}$	$\frac{1}{20}$	$\frac{1}{30}$	$\frac{1}{40}$	$\frac{1}{50}$	$\frac{1}{100}$	$\frac{1}{200}$

Table 3-2

tube no.	1	2	3	4	5	6	7	8	9	10	11	12	13	14
% conc. of NaCl	0.90	0.44	0.40	0.36	0.32	0.28	0.24	0.20	0.16	0.12	0.10	0.08	0.05	H_2O
volume of NaCl added (c.c.)	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4
volumne of RBC suspension (c.c.)	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
final volume (c.c.)	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
final conc. of NaCl (%)	0.90	0.532	0.50	0.468	0.436	0.404	0.372	0.34	0.308	0.276	0.26	0.244	0.22	0.18

Table 3-3

amount of blood given \ tube no. / final conc. of NaCl (%)	1	2	3	4	5	6	7	8	9	10	11	12	13	14
	0.90	0.532	0.50	0.468	0.436	0.404	0.372	0.34	0.308	0.276	0.26	0.244	0.22	0.18
$\frac{1}{500}$ ml.	5 +	5 +	4 +	3.5 +	3 +	3 +	2 +	−	−	−	−	−	−	−
$\frac{1}{1,000}$ ml.	4 +	4 +	3.5 +	3.5 +	3 +	2 +	+	−	−	−	−	−	−	−
$\frac{1}{1,500}$ ml.	3.5 +	3.5 +	3 +	2 +	2 +	+	+	−	−	−	−	−	−	−
$\frac{1}{2,000}$ ml.	3 +	3 +	2.5 +	1.5 +	+	⊥	⊥	−	−	−	−	−	−	−
$\frac{1}{2,500}$ ml.	2.5 +	2.5 +	2 +	2 +	1.5 +	+	⊥	−	−	−	−	−	−	−
$\frac{1}{5,000}$ ml.	1.5 +	+ >	+ >	+ >	+	⊥	⊥	−	−	−	−	−	−	−
$\frac{1}{10,000}$ ml.	+	+	+	+	⊥	⊥	−	−	−	−	−	−	−	−

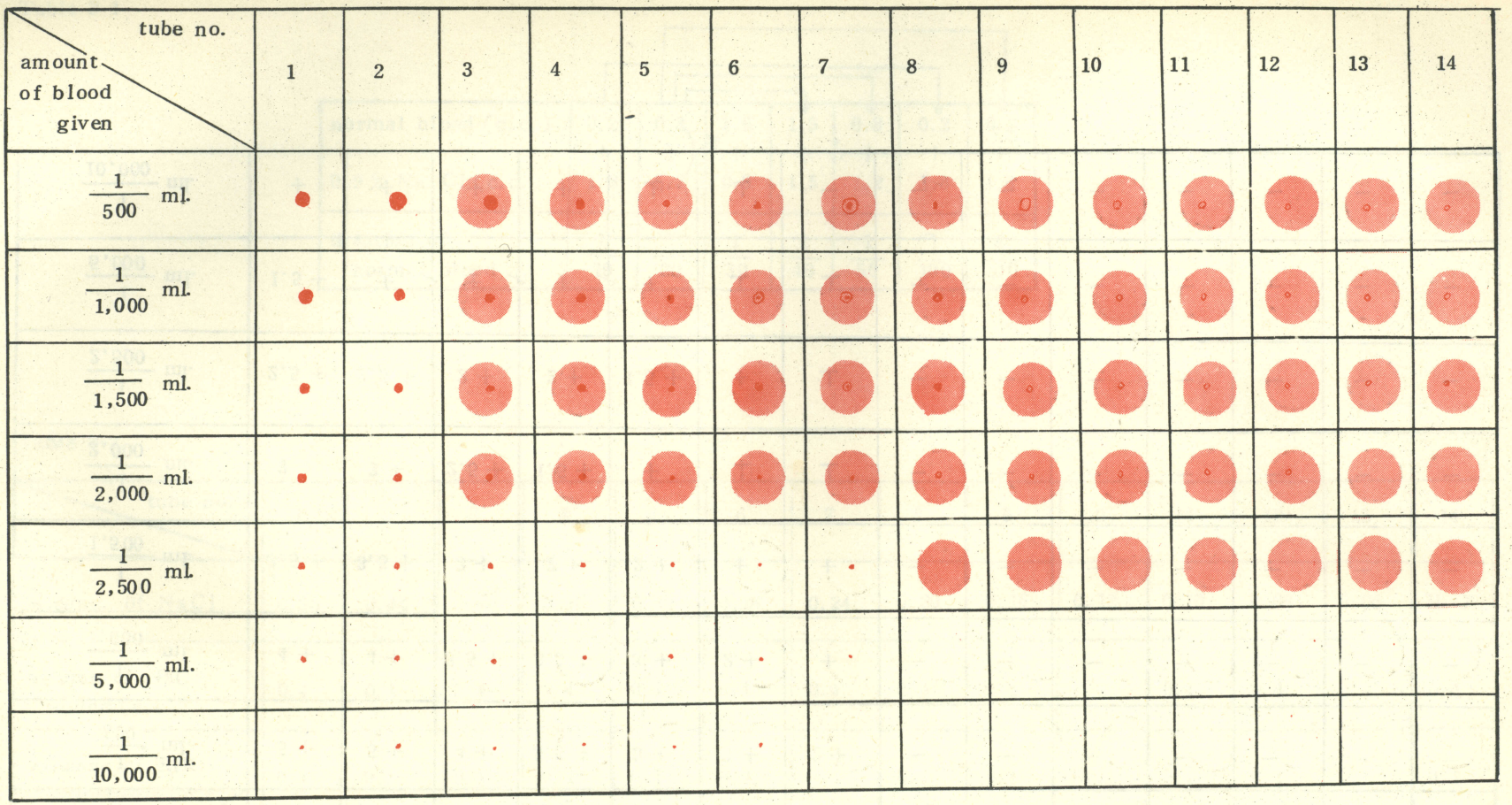


Fig6-2 The relative size of the packed RBC dot in the bottom of the hole. When the blood amount is large the red dot may become blurred and supernantant reveals reddish after a certain hemolysis.

Table 4-1

microtiter hole no.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
$\frac{1}{200}$ dilution of normal blood (ml.)	1.0	0.95	0.90	0.85	0.80	0.75	0.70	0.65	0.60	0.55	0.50	0.45	0.40	0.35	0.30	0.25	0.20	0.15	0.10	0.05	0.0
0.9 % NaCl (ml.)	0.0	0.05	0.10	0.15	0.20	0.25	0.30	0.35	0.40	0.45	0.50	0.55	0.60	0.65	0.70	0.75	0.80	0.85	0.90	0.95	1.0
Relative amount of RBC	100	95	90	85	80	75	70	65	60	55	50	45	40	35	30	25	20	15	10	5	0
Size of packed cell	4 +	4 +	4 +	4 +	4 +	4 +	4 +	4 +	4 +	4 +	3.5 +	3.5 +	3.5 +	3 +	3 +	2.5 +	2.5 +	2 +	+	⊥	—

Fig 7-

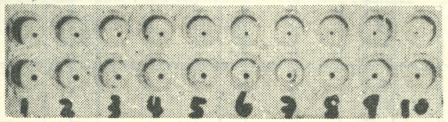


Table 4-2

microtiter hole no.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
$\frac{1}{1,000}$ dilution of normal blood (ml.)	1.0	0.95	0.90	0.85	0.80	0.75	0.70	0.65	0.60	0.55	0.50	0.45	0.40	0.35	0.30	0.25	0.20	0.15	0.10	0.05	0.0
0.9 % NaCl (ml.)	0.0	0.05	0.10	0.15	0.20	0.25	0.30	0.35	0.40	0.45	0.50	0.55	0.60	0.65	0.70	0.75	0.80	0.85	0.90	0.95	1.0
Relative amount of RBC	100	95	90	85	80	75	70	65	60	55	50	45	40	35	30	25	20	15	10	5	0
Size of packed cell	2 +	2 +	2 +	2 +	2 +	2 +	2 +	2 +	1.5 +	1.5 +	+	+	+	+	⊥	⊥	—	—	—	—	—

Fig 7-2

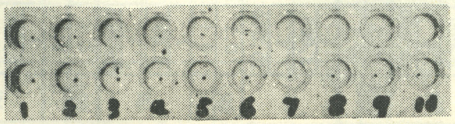


Table 5.

Case No.	method	* Amount of blood	reading method	NaCl%	1.00	0.95	0.90	0.85	0.80	0.75	0.70	0.65	0.60	0.56		0.52		0.48	
															0.532		0.50		0.468
1	Ma	0.1	P(%T)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0		0.0		0.0	
		0.1	V																
	Mi	1/2,000	V				4+								4+		4+		4+
		1/10,000	V				2+								2+		2+		2+
2	Ma	0.1	P(%T)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0		0.8		1.2	
		0.1	V																
	Mi	1/2,000	V				4+								4+		4+		3+
		1/10,000	V				2+								2+		2+		2+
3	Ma	0.1	P(%T)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.3	0.5		0.7		1.6 min	
		0.1	V																
	Mi	1/2,000	V				4+								4+		4+		3+
		1/10,000	V				2+								2+		2+		1.5+
4	Ma	0.1	P(%T)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0		0.0		0.2	
		0.1	V																
	Mi	1/2,000	V				4+								4+		4+		4+
		1/10,000	V				2+								2+		2+		2+
5	Ma	0.1	P(%T)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0		0.0		0.0	
		0.1	V																
	Mi	1/2,000	V				4+								4+		4+		4+
		1/10,000	V				2+								2+		2+		2+
6	Ma	0.1	P(%T)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0		0.0		0.0 min	
		0.1	V																
	Mi	1/2,000	V				4+								4+		4+		4+
		1/10,000	V				2+								2+		2+		2+
7	Ma	0.1	P(%T)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0		0.3		1.0 min	
		0.1	V																
	Mi	1/2,000	V				4+								4+		3+		3+
		1/10,000	V				2+								2+		2+		+
8	Ma	0.1	P(%T)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0		0.0		0.0	
		0.1	V																
	Mi	1/2,000	V				4+								4+		4+		3+
		1/10,000	V				2+								2+		2+		2+
9	Ma	0.1	P(%T)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0		0.0		0.8 min	
		0.1	V																
	Mi	1/2,000	V				4+								4+		4+		3+
		1/10,000	V				2+								2+		2+		1.5+
10	Ma	0.1	P(%T)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0			0.0		0.9 min	
		0.1	V																
	Mi	1/2,000	V				4+								4+		4+		3+
		1/10,000	V				2+								2+		2+		+

*: oxalated blood

0.44			0.40		0.36		0.32		0.28				0.24		0.20		H_2O
	0.436	0.404		0.372		0.34		0.308		0.276	0.26	0.244		0.22		0.18	
0.2			4.3		29.2		85.6		92.1				93.2		94.5		100
min									max.								
	3 +	29		2 +		+		—		—	—	—		—		—	
	2 +	+		+		—		—		—	—	—		—		—	
3.1			13.7		52.6		88.6		93.2				95.8		97.1		100
min									max.								
	2 +	2 +		+		+		—		—	—	—		—		—	
	+	+		—		—		—		—	—	—		—		—	
4.0			14.1		49.1		76.6		91.2				93.8		93.8		100
									max.								
	2 +	+		+		—		—		—	—	—		—		—	
	+	—		—		—		—		—	—	—		—		—	
2.8			12.2		54.2		77.9		85.6				89.6		96.9		100
min							max.										
	3 +	2 +		+		—		—		—	—	—		—		—	
	2 +	+		—		—		—		—	—	—		—		—	
0.6			5.0		21.5		65.8		79.3				89.3		97.3		100
min									max.								
	4 +	3 +		2 +		+		+		—	—	—		—		—	
	2 +	+		—		—		—		—	—	—		—		—	
3.0			15.0		62.4		79.7		94.9				97.4		100		100
							max.										
	3 +	2 +		+		—		—		—	—	—		—		—	
	2 +	+		—		—		—		—	—	—		—		—	
6.4			26.4		75.0		90.8		94.4				98.5		100		100
							max.										
	3 +	2 +		+		—		—		—	—	—		—		—	
	+	+		—		—		—		—	—	—		—		—	
1.4			5.9		33.5		81.2		89.5				97.6		98.8		100
min									max.								
	3 +	3 +		2 +		+		—		—	—	—		—		—	
	2 +	+		+		—		—		—	—	—		—		—	
3.5			30.8		79.6		91.7		96.7				97.7		98.8		100
							max.										
	2 +	+		—		—		—		—	—	—		—		—	
	+	0.5 +		—		—		—		—	—	—		—		—	
6.8			26.3		71.2		92.6		98.8				100		100		100
							max.										
	3 +	2 +		+		—		—		—	—	—		—		—	
	0.5 +	0.5 +		—		—		—		—	—	—		—		—	

Table 6-1

Case No.		1	2	3	4	5	6	7	8	9	10	ave
Min. H. (macro P)	5 %	0.39	0.42	0.43	0.42	0.40	0.43	0.44	0.40	0.43	0.45	0.421
	10 %	0.38	0.40	0.41	0.41	0.37	0.41	0.42	0.38	0.42	0.42	0.402
% of NaCl sol. with the Smallest RBC dot.		0.372	0.404	0.436	0.404	0.404	0.404	0.404	0.372	0.404	0.404	0.4008
max. H. (macro P)	90 %	0.3	0.31	0.29	0.26	0.25	0.30	0.32	0.28	0.32	0.32	0.295
	95 %	0.2	0.25	0.20	0.22	0.20	0.28	0.29	0.25	0.29	0.30	0.248
% of NaCl of the first tube with no RBC dot		0.308	0.308	0.340	0.340	0.276	0.340	0.340	0.308	0.372	0.340	0.3368
Relation of % of NaCl Sol. with the smallest RBC dot to min. H. (5% & 10%) read on the curve	5 %											
	10 %											
Relation of % of NaCl Sol. of the first tube with no RBC dot to max. H. (90% & 95%) read on the curve	90 %											
	95 %											

Comparision of minimal hemolysis of macro-photometry method & micro-visual method:

+ : point of minimal hemolysis is to be at the last ⊕

↶+ : point of minimal hemolysis is to be at the last ⊕

+↷ : point of minimal hemolysis is to be one tube right to the last ⊕

↑+ : point of minimal hemolysis is between the last two ⊕

↑↷+ : point of minimal hemolysis is to be between last two before the last ⊕

Comparision of max imal hemolysis of macro-photometry method & micro-visual method:

− : point of maximal hemolysis is to be at the first ⊖ tube

↑− : point of maximal hemolysis is to be between ⊕ tube & the first ⊖ tube

−↑ : point of maximal hemolysis is to be between first two ⊖ tubes

−∩ : point of maximal hemolysis is to be at the 2nd ⊖ tube

−ᴨ : point of maximal hemolysis is to be at the 3rd ⊖ tube

−ᴨ↑ : point of maximal hemolysis is to be between the 2nd ⊖ tube & the third ⊖ tube

−ᴨ↷ : point of maximal hemolysis is to be between the 3rd ⊖ tube & the 4th ⊖ tube

−ᴍ : point of maximal hemolysis is to be at the 4th ⊖ tube

−ᴍ : point of maximal hemolysis is to be at the 5th ⊖ tube

Table 6-2

Case no.		1	2	3	4	5	6	7	8	9	10	ave
% of NaCl sol with the smallest RBC dot		0.372	0.404	0.436	0.404	0.404	0.404	0.404	0.372	0.404	0.404	0.4008
Minimal hemolysis (macro-photometry method)	5 %	0.390	0.420	0.43	0.42	0.40	0.43	0.44	0.40	0.43	0.45	0.421
		0.018	0.016	− 0.006	0.016	− 0.004	0.026	0.036	0.028	0.026	0.046	0.0202
	10 %	0.38	0.40	0.41	0.41	0.37	0.41	0.42	0.38	0.42	0.42	0.402
		0.008	− 0.004	− 0.025	0.006	− 0.034	0.006	0.016	0.008	0.016	0.016	0.0013
% of NaCl of the first tube with no RBC dot		0.308	0.308	0.340	0.340	0.276	0.340	0.340	0.308	0.372	0.340	0.3368
maximal hemolysis (macro-photometry method)	90 %	0.3	0.31	0.29	0.26	0.25	0.30	0.32	0.28	0.32	0.32	0.295
		− 0.008	+ 0.002	− 0.050	− 0.080	− 0.026	− 0.040	− 0.020	− 0.028	− 0.052	− 0.020	− 0.0322
	95 %	0.20	0.25	0.20	0.22	0.20	0.28	0.29	0.25	0.29	0.30	0.248
		− 0.108	− 0.058	− 0.140	− 0.120	− 0.076	− 0.060	− 0.050	− 0.058	− 0.082	− 0.040	− 0.0792

Table 6-3

Comparision of minimal & maximal hemolysis value between two different method : macro-visual & micro-visual method.

Case no.		1	2	3	4	5	6	7	8	9	10
Minimal hemolysis	macro P*1	0.39	0.42	0.43	0.42	0.40	0.43	0.44	0.40	0.43	0.45
	macro V	0.44	0.44	0.48	0.44	0.44	0.44	0.48	0.44	0.48	0.48
	micro V	0.404	0.436	0.468	0.436	0.436	0.436	0.436	0.404	0.436	0.436
Minimal hemolysis	macro P*2	0.30	0.31	0.29	0.26	0.25	0.30	0.32	0.28	0.32	0.32
	macro V	0.28	0.28	0.28	0.32	0.28	0.32	0.32	0.28	0.32	0.32
	micro V	0.276	0.276	0.308	0.308	0.26	0.308	0.308	0.276	0.34	0.308

*1 : 5 % hemolysis is regarded as minimal hemolysis

*2 : 90 % hemolysis is regarded as maximal hemolysis

Fig. 10
Case No. 3

macroP { min H 0.43 %
max H 0.29 %

microV { min H 0.468 %
max H 0.308 %

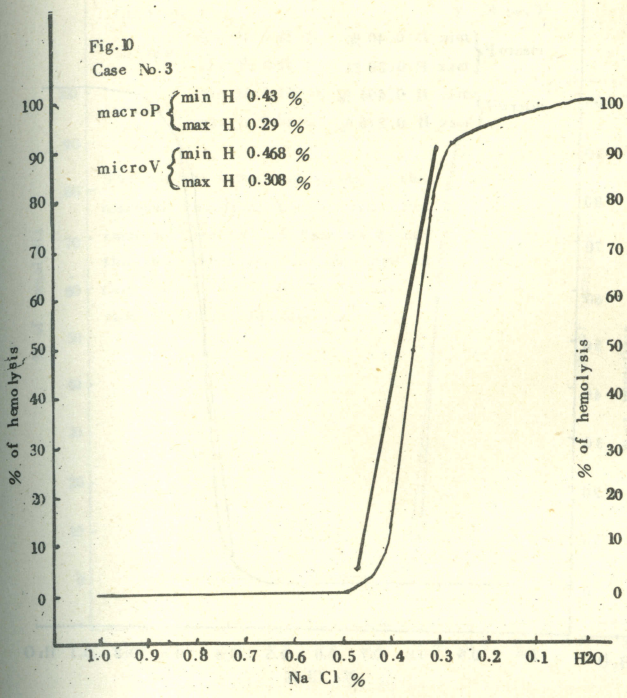


Fig. 11
Case No. 4

macroP { min H 0.42 %
max H 0.26 %

microV { min H 0.436 %
max H 0.308 %

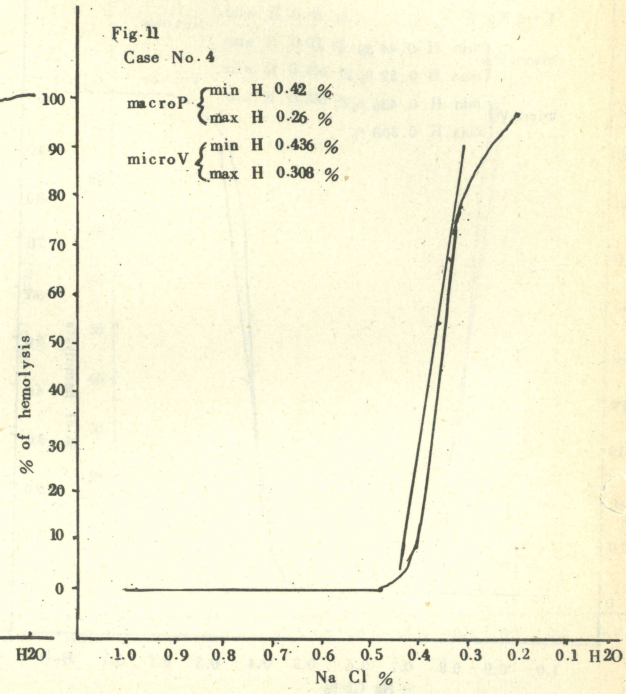


Fig. 8
Case No. 1

macroP { min H 0.39 %
max H 0.30 %

microV { min H 0.404 %
max H 0.276 %

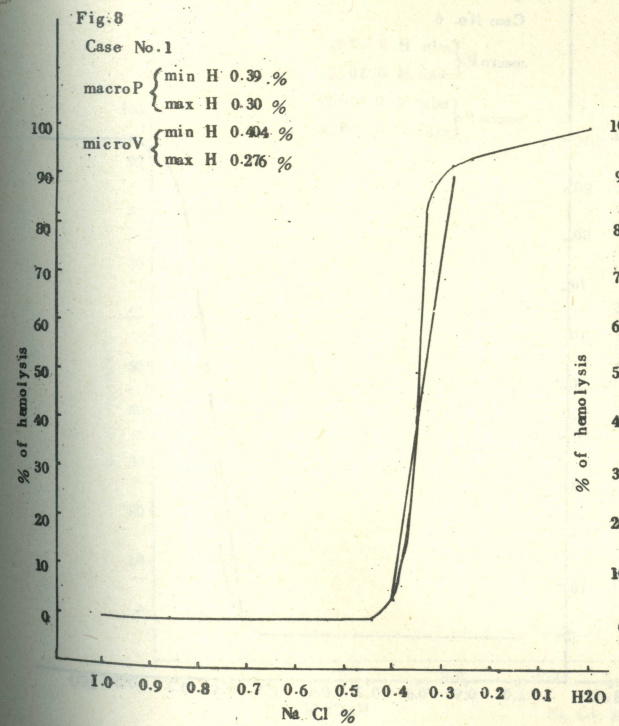


Fig. 9
Case No. 2

macroP { min H 0.42 %
max H 0.31 %

microV { min H 0.436 %
max H 0.276 %

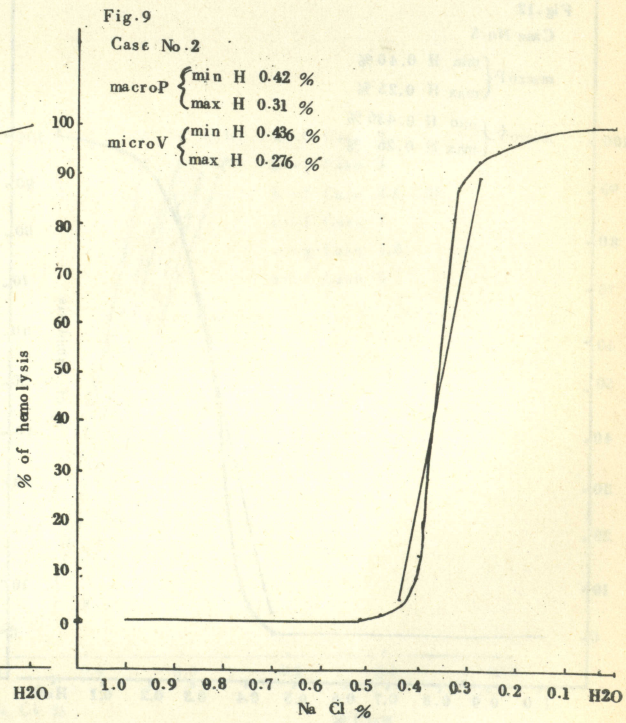


Fig. 14

Case No. 7

macro P { min H 0.44 %
max H 0.32 %

micro V { min H 0.436 %
max H 0.308 %

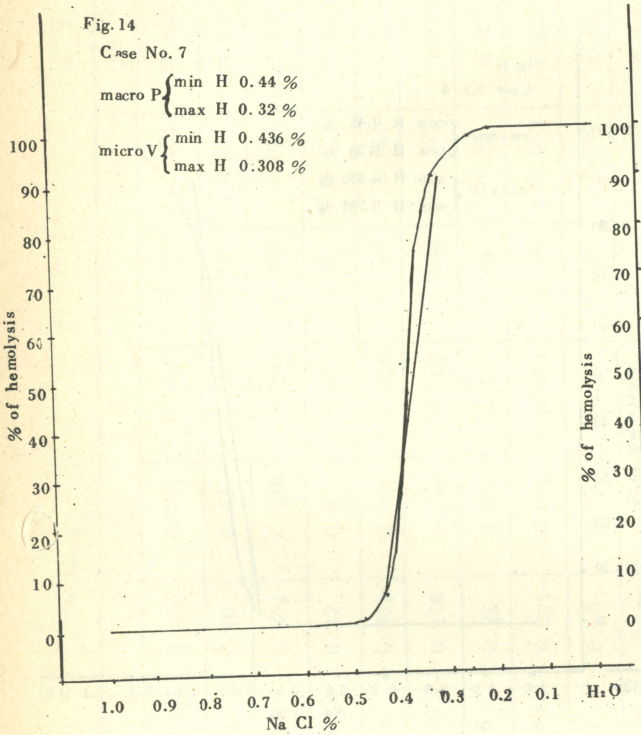


Fig. 15

Case No. 8

macro P { min H 0.40 %
max H 0.28 %

micro V { min H 0.404 %
max H 0.276 %

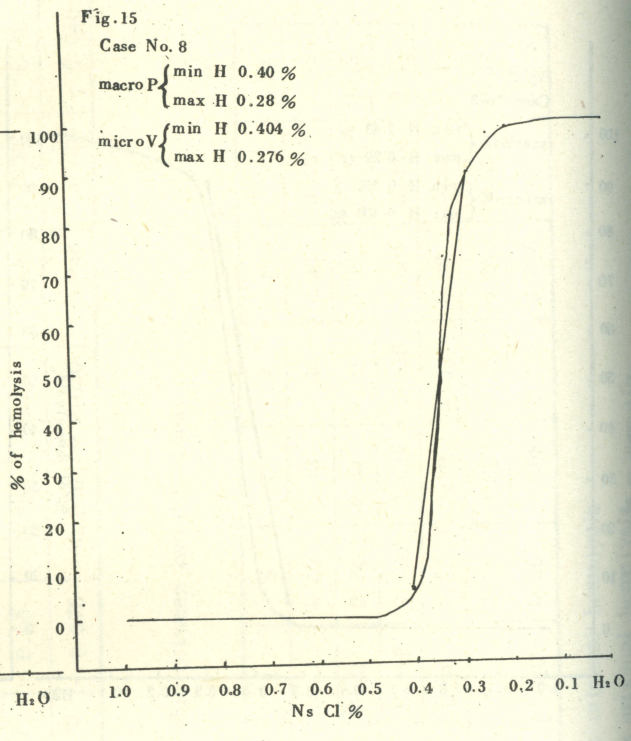


Fig. 12

Case No. 5

macro P { min H 0.40 %
max H 0.25 %

micro V { min H 0.436 %
max H 0.26 %

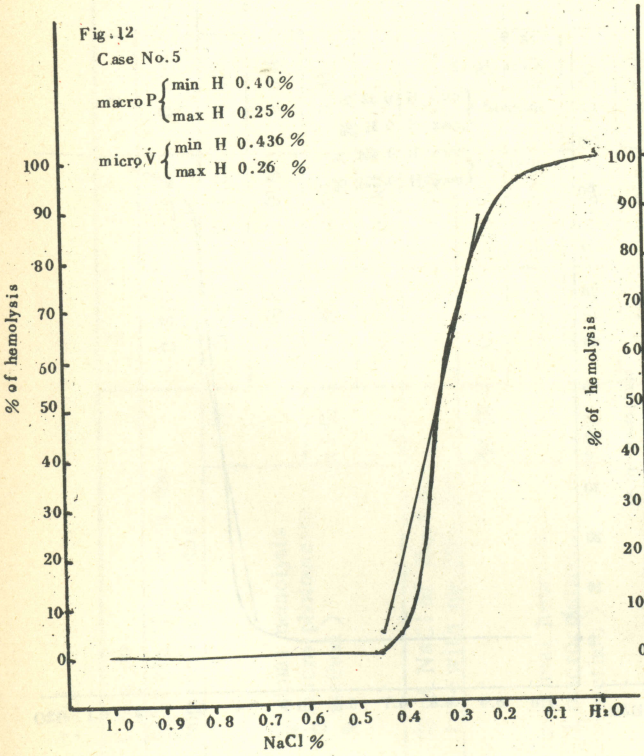


Fig. 13

Case No. 6

macro P { min H 0.43 %
max H 0.30 %

micro P { min H 0.436 %
max H 0.308 %

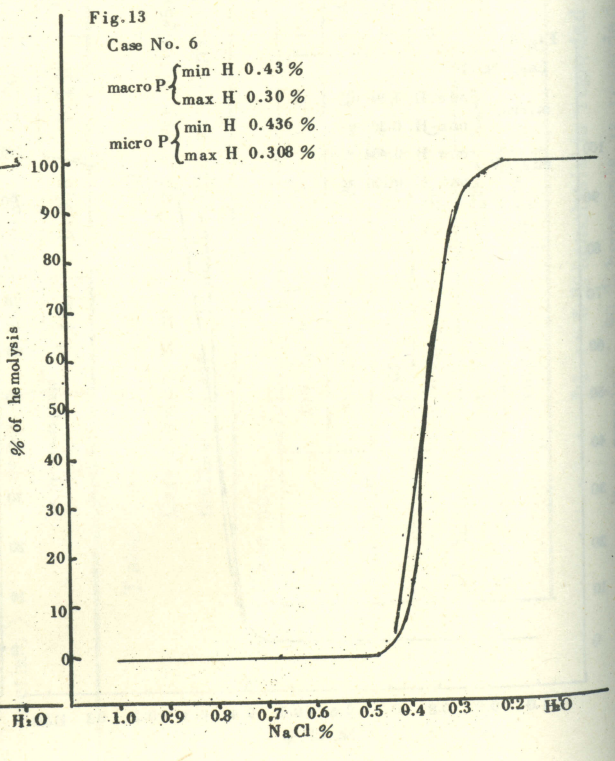


Fig. 16

Case No 9

macroP min H 0.43 %
max H 0.32 %

microV min H 0.436 %
max H 0.34 %

The minimal hemolysis points & the maximal hemolysis points are the same in Case no. 1 & Case no. 8, and the two points are also the same in Case no. 4, 6, 7, & 10. so the straight lines are only six

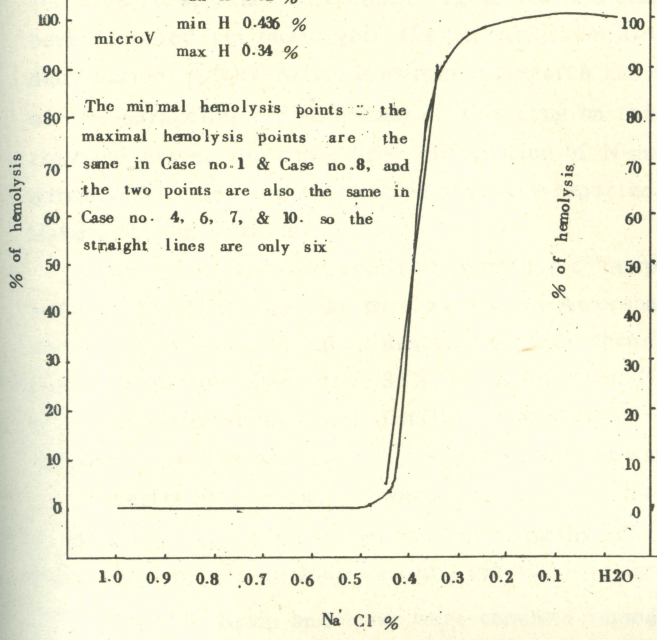


Fig. 17

Case No. 10

macroP min H 0.45 %
max H 0.32 %

microV min H 0.436 %
max H 0.308 %

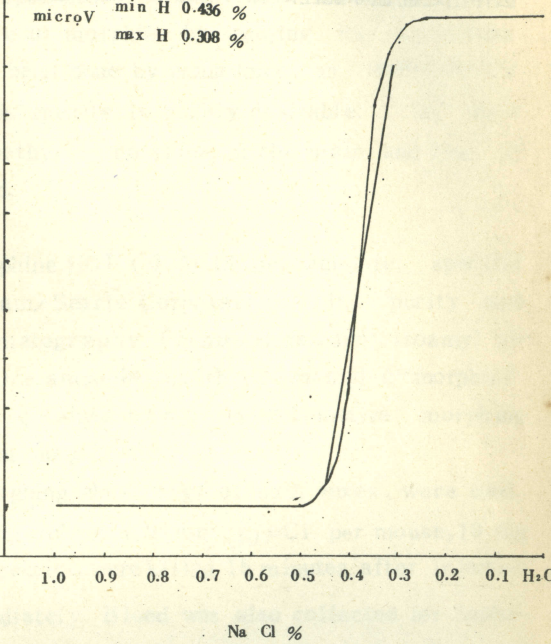
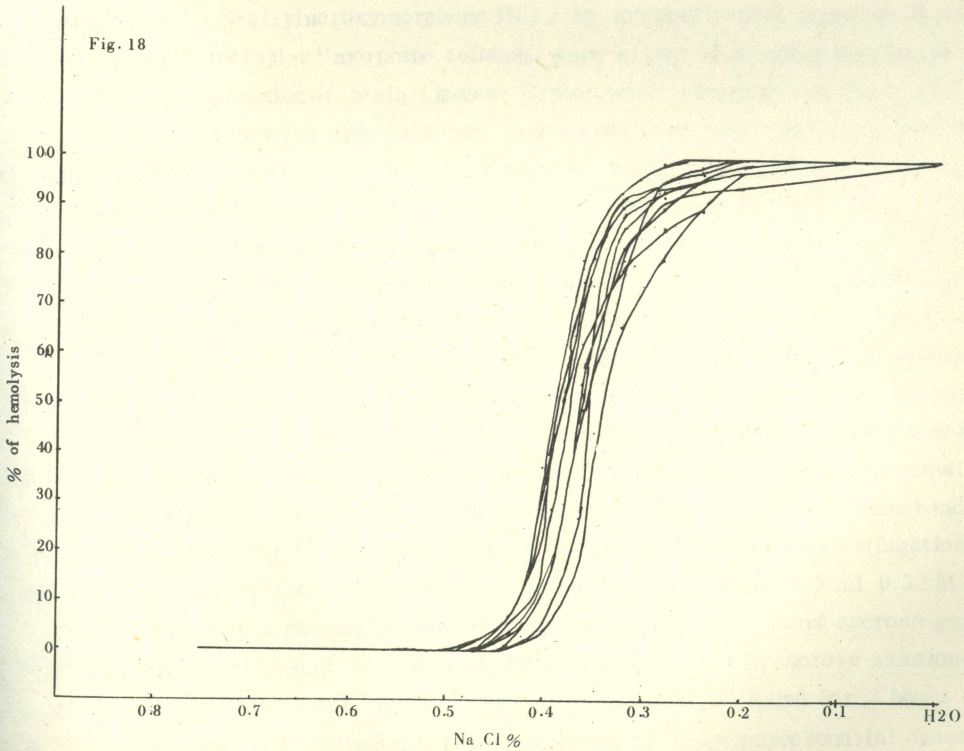


Fig. 18



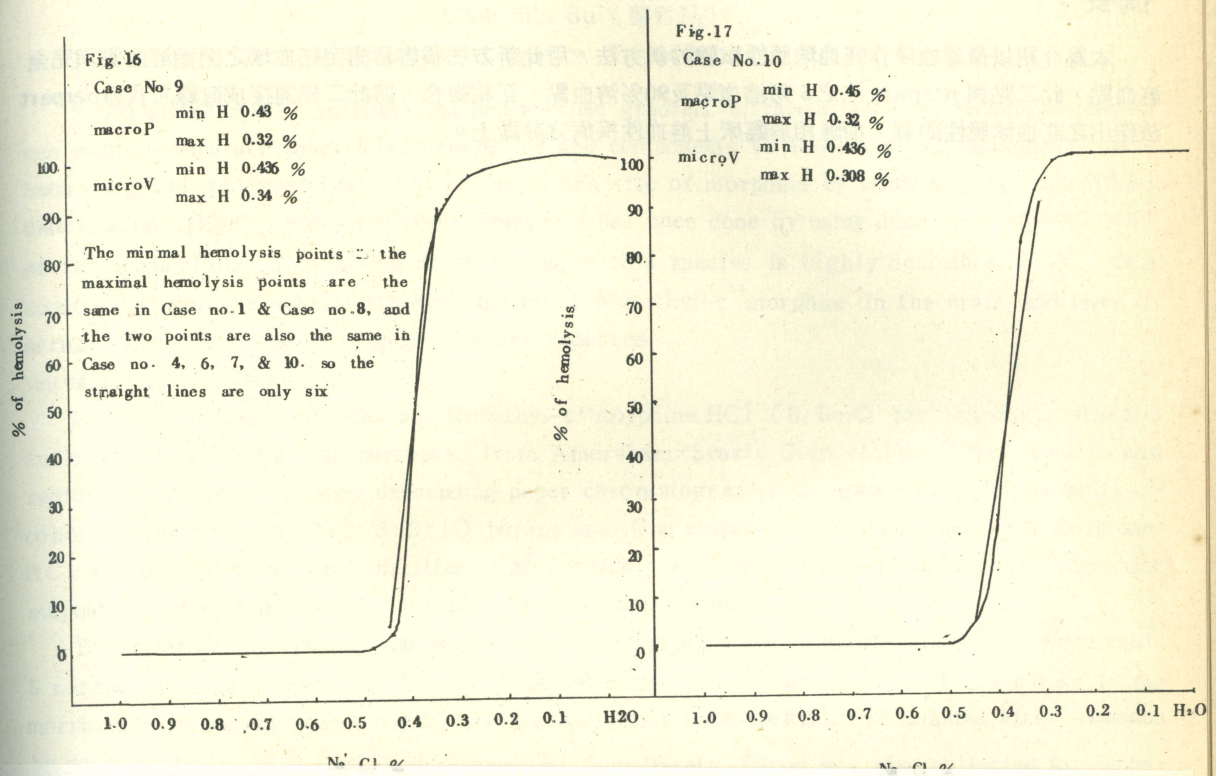


Fig.16
Case No 9
macroP min H 0.43 %
max H 0.32 %
microV min H 0.436 %
max H 0.34 %
The minimal hemolysis points & the maximal hemolysis points are the same in Case no.1 & Case no.8, and the two points are also the same in Case no. 4, 6, 7, & 10. so the straight lines are only six
% of hemolysis
100 90 80 70 60 50 40 30 20 10 0
1.0 0.9 0.8 0.7 0.6 0.5 0.4 0.3 0.2 0.1 H2O
Na Cl %
Fig.17
Case No.10
macroP min H 0.45 %
max H 0.32 %
microV min H 0.436 %
max H 0.308 %
% of hemolysis
100 90 80 70 60 50 40 30 20 10 0
1.0 0.9 0.8 0.7 0.6 0.5 0.4 0.3 0.2 0.1 H2O
Na Cl %

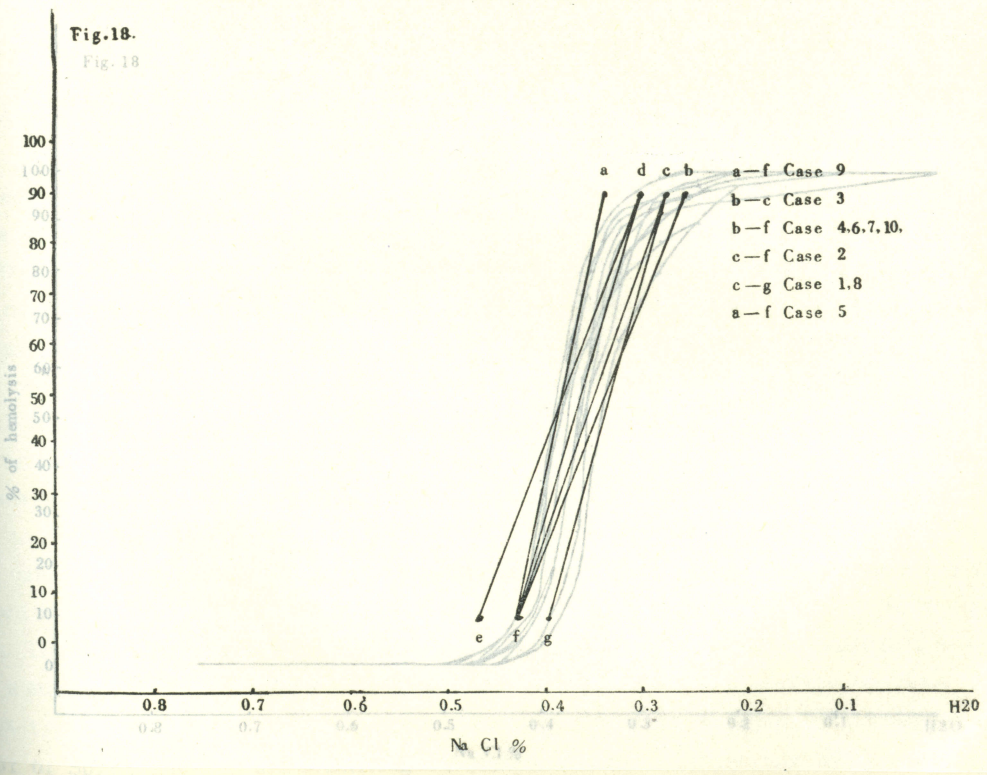


Fig.18.
% of hemolysis
100 90 80 70 60 50 40 30 20 10 0
a d c b
e f g
a—f Case 9
b—c Case 3
b—f Case 4.6.7.10.
c—f Case 2
c—g Case 1.8
a—f Case 5
0.8 0.7 0.6 0.5 0.4 0.3 0.2 0.1 H20
Na Cl %

用微量血液作紅血球脆性試驗

摘要：

本篇介紹以微量血液作紅血球脆性試驗的新方法，用此新方法很容易測定紅血球之開始溶血點與完全溶血點，此二點與 parpart 法之5％溶血點及90％溶血點，互相吻合，將此二點連接成直線可代替parpart法作出之紅血球脆性曲線，而應用於臨床上溶血性疾病之診斷上。