

## 1-20

# *Osmotic Fragility of Red Blood Cells* (Syn: Osmotic Resistance of Red Blood Corpuscles)

**STUDENT OBJECTIVES**

After completing this experiment, you should be able to:

1. Define the osmotic fragility of red cells and describe the utility of this test.
2. Define the terms osmosis, exosmosis, and endosmosis.
3. Explain how hemolysis of RBCs occurs when they are exposed to hypotonic saline.
4. Explain the effect of hypertonic saline on red cells.
5. Name the conditions in which fragility of red cells is increased and decreased.

**Relevance**

In certain hemolytic anemias, the red cells become more fragile, i.e., they are likely to burst and release their hemoglobin into the plasma. The osmotic fragility test assesses their ability to withstand hypotonic saline without bursting. It is employed as a screening test for hemolytic anemias.

**PRINCIPLE**

The normal red cells can remain suspended in normal saline (0.9% NaCl solution) for hours without rupturing or any change in their size or shape. But when they are placed in decreasing strengths of hypotonic saline, they imbibe water (due to osmosis) and finally burst. The ability of RBCs to resist this type of hemolysis can be determined quantitatively.

**APPARATUS AND MATERIALS**

1. Wood or metal test tube rack with 12 clean, dry, 7.5 cm × 1.0 cm glass test tubes. •Glass marking pencil. •Glass dropper with a rubber teat.
2. Sterile swabs moist with alcohol. •2 ml syringe with needle.
3. Freshly prepared 1 percent sodium chloride solution. •Distilled water.

**PROCEDURES**

1. Number the test tubes from 1 to 12 with the glass-marking pencil and put them in the rack.
2. Using the glass dropper, place the varying number of drops of 1% saline in each of the 12 test tubes as shown in Table 1.6. Then, after thorough rinsing of the same dropper with distilled water, add the number of drops of distilled water to each of the 12 tubes, as shown in Table 1.6.

Mix the contents of each test tube by placing a thumb over it and inverting it a few times. Mark the tonicity of saline on each of the test tubes. Note that tube # 1 contains normal saline, which is isotonic with plasma, while tube # 12 contains only distilled water which has no tonicity.

3. Draw 2 ml of blood from a suitable vein and gently eject one drop of blood into each of the 12 tubes. (The blood may be put into a container of anti-coagulant, and a drop can be put into each tube with a pipette). Mix the contents gently by placing a thumb over it and inverting the tube only once.
4. Leave the test tubes undisturbed for one hour. Then observe the extent of hemolysis in each tube by holding the rack at eye level, with a white paper sheet behind it.

**OBSERVATIONS AND RESULTS**

While judging the degree or extent of hemolysis from the depth of the red color of supernatant saline, tube # 1 (normal saline), and tube # 12 (distilled water) will act as controls, i.e. no hemolysis in normal saline (# 1) and complete hemolysis in distilled water (# 12).

- a. The test tubes in which no hemolysis has occurred, the RBCs will settle down and form a red dot (mass) at the bottom of the tube, leaving the saline above clear.

**Table 1.6:** Preparation of saline solutions for testing the osmotic fragility of red cells

Test tube number	1	2	3	4	5	6	7	8	9	10	11	12
No. of drops of 1% NaCl	22	16	15	14	13	12	11	10	9	8	7	0
No. of drops of distilled water	3	9	10	11	12	13	14	15	16	17	18	25
Tonicity strength of NaCl (in%)	0.88	0.64	0.60	0.56	0.52	0.48	0.44	0.40	0.36	0.32	0.28	0
Note: Use the same dropper, after thorough rinsing each time, for measuring saline and distilled water. This will ensure that the volume of all drops is equal for all test tubes.												

- b. If there is some hemolysis, the saline will be tinged red with Hb, with the unruptured cells forming a red dot at the bottom. The color of the saline will be seen to be increasingly deeper with decreasing tonicity of saline.
- c. The test tubes in which there is complete hemolysis, the saline will be equally deep red with no red cells at the bottom of these tubes.

**Results.** Carefully observe each tube for depth of red color of the supernatant and the mass of red cells at the bottom.

- Note the start of hemolysis (also called onset of fragility) and record the test tube number. Express your result in % saline.
- Note the start of complete hemolysis, i.e. the test tube in which there are no red cells at the bottom (hemolysis will be complete below this saline strength). Express your result in % saline.

Hemolysis begins in ..... % saline.

Hemolysis is complete in ..... % saline.

If there is doubt about the presence of intact RBCs at the bottom of a test tube, the solution can be centrifuged and the sediment examined under the microscope.

### Normal Range of Fragility

Normally, hemolysis begins in about 0.48% saline (tube # 6 in this case). No cells hemolyze in solutions of 0.5% saline and above.

Hemolysis is complete at about 0.36 % saline (tube # 9). It is also complete in tubes 10, 11, and 12.

### Note

When the test is done on a patient, it is always checked against a normal sample of blood, which is tested on a separate series of saline solutions.

- When red cells become more fragile, hemolysis may begin at about 0.64% saline and be complete at about 0.44% saline.
- When red cells are less fragile, hemolysis starts and is complete at lower strengths of saline.

### Modified Experiment

In this test, the red cell fragility is tested by counting the cells in a hemocytometer, using 0.45% saline for diluting the blood in one pipette, and using Hayem's fluid for diluting blood in a second pipette. Both pipettes are shaken for about 2 minutes and counts are made from both pipettes. The percent of red cells hemolysed in 0.45% saline is thus determined. Less than 20% of normal RBCs are hemolyzed by this method. In hereditary spherocytosis, the abnormal increase in fragility may cause hemolysis of more than 70% of red cells.

### Precautions

1. Use the same dropper, after thorough rinsing each time, for measuring saline and distilled water.
2. The test tubes should not be shaken vigorously after adding blood, because this is likely to cause mechanical hemolysis.
3. The test tubes should be left undisturbed for one hour before making the observations.