**ANTICOAGULANTS**

EDTA and sodium citrate remove calcium, which is essential for coagulation. Heparin binds to antithrombin, thus inhibiting the interaction of several clotting factors.

EDTA is used for blood counts; sodium citrate is used for coagulation testing and erythrocyte sedimentation rate (ESR). For better long-term preservation of red cells for certain tests and for transfusion purposes, citrate is used in combination with dextrose in the form of acid–citrate–dextrose (ACD), citrate–phosphate–dextrose (CPD).

***Ethylenediaminetetra-Acetic Acid***

The sodium and potassium salts of EDTA are powerful anticoagulants, and they are especially suitable for routine haematological work. EDTA acts by its chelating effect on the calcium molecules in blood. To achieve this requires a concentration of 1.2mg per ml of blood. The dipotassium salt is very soluble and is to be preferred on this account to the disodium salt, which is considerably less soluble.

The dilithium salt of EDTA is equally effective as an anticoagulant, and its use has the advantage that the same sample of blood can be used for chemical investigation. However, it is less soluble than the dipotassium salt .

The tripotassium salt produces some shrinkage of red cells, which results in a 2-3% decrease in PCV within 4 hours of collection, followed by a gradual increase in (MCV). By contrast, there are negligible changes when the dipotassium salt is used.

Excess of EDTA, irrespective of which salt, affects both red cells and leucocytes, causing shrinkage and degenerative changes. EDTA in excess of 2 mg/ml of blood may result in a significant decrease in PCV by centrifugation and increase in (MCHC). The platelets are also affected; excess of EDTA causes them to swell and then disintegrate, leading to an artificially high platelet count because the fragments are large enough to be counted as normal platelets. Care must therefore be taken to ensure that the correct amount of blood is added and that by repeated inversions of the container the anticoagulant is thoroughly mixed in the blood added to it. Blood films made from EDTA blood may fail to demonstrate basophilic stippling of the red cells in lead poisoning.

EDTA has also been shown to cause leucoagglutination affecting both neutrophils and lymphocytes, and it is responsible for the activity of a naturally occurring antiplatelet autoantibody, which may sometimes cause platelet adherence to neutrophils in blood films.

***Trisodium Citrate***

For coagulation studies 9 volumes of blood are added to 1 volume of sodium citrate solution . This ratio of anticoagulant to blood is critical as osmotic effects and changes in free calcium ion concentration affect coagulation test results.

For the ESR, 4 volumes of blood are added to 1 volume of the sodium citrate solution and immediately well mixed with it. The mixture is taken up in a Westergren tube.

***Heparin***

Lithium or sodium salt of heparin at a concentration of 10-20 iu per ml of blood is a commonly used anticoagulant for chemistry, gas analysis, and emergency tests. It does not alter the size of the red cells. It is thus the best anticoagulant for osmotic fragility tests and is suitable for immunophenotyping.

However, heparin is not suitable for blood counts because it often induces platelet and leucocyte clumping.It also should not be used for making blood films because it gives a blue colouration to the background. It inhibits enzyme activity, and it should not be used in the study of PCR with restriction enzymes.