Figure 28-4 Blood from horseshoe crab after centrifugation. (Horseshoe crab blood color is blue due to copper being the metal in the oxygen carrying hemocyanin, as opposed to iron in hemoglobin. White pellets at bottom contain the amebocytes.) *Source*: Courtesy of Associates of Cape Cod, Inc.

is extremely sensitive to heat and even in the lyophilized state must be stored in the freezer (16). Upon reconstitution, LAL has a shelf life of one month's storage at freezing conditions.

LAL Reaction Mechanism

Endotoxin or a suitably prepared lipid-A derivative of endotoxin activates a proenzyme of LAL having a molecular weight of 150,000. Activation also depends on the presence of divalent metal cations such as calcium, manganese, or magnesium. It has been shown that the sensitivity of the LAL assay for endotoxin detection can be increased 10 to 30 times by using LAL reagent containing 50 mM magnesium (17).

The activated proenzyme, related to the serine protease class containing such enzymes as thrombin, trypsin, and factor Xa, subsequently reacts with a lower molecular weight protein fraction (MW = 19,000–25,000) contained also in the LAL substance. The lower molecular weight fraction, called coagulogen, is cleaved by the proenzyme into a soluble and insoluble subunit. The insoluble subunit appears as a solid clot, a precipitate, or a turbid solution, depending on the amount of insoluble coagulogen by-product formed.

Therefore, the coagulation reaction requires three factors in addition to endotoxin. These three factors—a clotting enzyme, clottable protein (coagulogen), and certain divalent cations—are found in the LAL reagent. A schematic representation of the LAL reaction mechanism is found in Figure 28-5 (18).

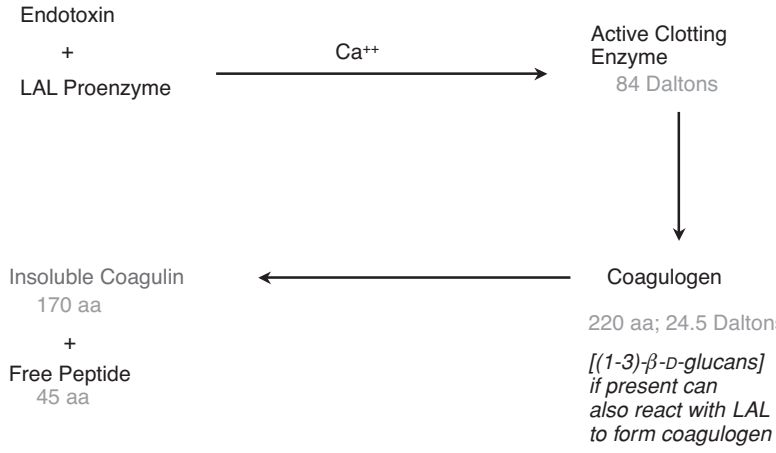

Figure 28-5 LAL reaction mechanism (simplified).