The term “eryptosis” has been introduced a few years ago by Florian Lang to describe a set of mechanisms at work in RBCs in response to various stresses, that closely resembles classical apoptosis pathways. Common to all studies is a large influx of ionic calcium as a very initial event in RBC injury [31–38]. Reasons for this calcium intake are largely unknown, but altered activity of non-specific (NS) cation channels is often mentioned as a possible cause. As shown in [39], one documented activation of non-specific cation channelscanoccurthroughprostaglandinE2stimulation,that can be synthesized by the phospholipase A2/cyclooxygenase classical studies [23], demonstrated an accumulation of oxidatively damaged membrane proteins throughout storage [24], as well asanaccumulationandclusteringofhaemoglobin dimers at the internal side of the plasma membrane [25]. Membranereorganisation is accompanied in some cases by phosphatidylserine (PS) exposure to the outer leaflet of the plasma membrane. Normally PS is sequestered to the inner leaflet oftheplasmamembranebyacomplexsystemcomposed offlippase,floppaseandscamblase.Thereisnoclearconsensus if a fraction of stored erythrocytes exhibits this PS exposure: some authors observed PS exposure [26], typicallyby flow cytometric analysis of Annexinbinding,whereasothersdid not [27]. In most cases, PS exposure was observed after inducing specific stresses in vitro that are hypothesised to mimic RBC senescence, but the relevance of these observations to spontaneousRBCalterationsduringstorageremainsquestionable[28]. Lastly, Sparrow et al. studied the bindingof RBCs tovarious lectins throughout storage, and found that various lectins bound differently to RBCs depending on the storage duration: whereas the binding of lectins specific for (α-2,3)-linked sialic acids showed no difference in the course of storage, the binding of lectins to N-acetylglucosamine and (α-2,6)-linked sialic acids showed marked difference between the beginning of storage(day1)andtheendofstorage(day42)[29].Thestudy was further complicated by the difference in lectin binding according to the age of erythrocytes at collection: whereas (COX) system in response, for example, to an osmotic shock. Synthesis of PGE2hasbeenreportedtobeinducedbyexposure to high osmolarity [39]; in this case, PGE2 may activate NS cation channels whether intracellularly, or after secretion through the multidrug resistant protein 4. The same stimulus results in the synthesis of the platelet-activating factor (PAF) that can activate sphingomyelinase, thus producing ceramide and disrupting the RBC membrane organisation [40], and also sensitize Gardos channels to Ca2+ activation. Elevation of intracellular Ca2+ concentration leads to direct activation of the Gardos channels and thus to loss of K+,Cl- and water, activation of scramblase, which perturbs the normal asymmetry of RBC membrane and leads to phosphatidylserine externalization [41,42]. Intracellularly, Ca2+ influx also activates calpains [43,44], a family of cysteine proteases that is physiologically inhibited by the binding of calpastatin. Uponcalpastatinreleaseandcalciumbinding,calpainsbindto the inner side of the membranes, where they degrade cytoskeletal proteins, such as proteins 2.1 and 4.1 and spectrin. Calcium increase also directly activates transglutaminase 2 (TG2), which crosslinks proteins of the cytoskeleton [45,46]. The combinedactionofcalpaindegradationandTG2is most probably responsible for the loss of cytoskeleton plasticity and cellular deformability. Of importance is also theroleofcalciuminthedisruptionoftheinteractionbetween Band3, amajormembraneproteinandanionexchanger,with protein tyrosine phosphatase 1B (PTP1B); in

physiological conditions, PTP1B binds to the cytoplasmic domain of Band 3 and maintains the phosphorylation status of two tyrosines [47–49]. When the interaction is disrupted by calcium, SRC kinase syk and kinases lyn remain unchallenged and the phosphorylationstatusof thecytoplasmicdomainofBand3is modified. The role of these tyrosine phosphorylations is not fully understood, but as the cytoplasmic domain of Band 3 plays multiple roles in the anchorage with the cytoskeleton (through interaction with protein 4.2 and spectrin), binding of glycolytic enzymes, and binding of haemoglobin, it is highly probable that modification of the phosphorylation status of the cytoplasmic domain of Band 3 serves as a signaling event (see below) [50–55].

Theeryptosismodelaccountswellfortoxicologicalstudies, whereRBCdegradationoccursfollowingexposuretoinorganic ions such as gold, cadmium [56], selenium [57], tin [58]..., or various drugs such as zidovudine [59], cyclosporine , azathioprine, PPAR agonists, cisplatin [60]... It also correlates well with observed RBC response to osmotic stress [61] and energy depletion [62] (Fig. 4). However,iftheeryptosismodelexplainsthelossofcellular deformability, possible glycolysis alteration through modified interactions with the cytosolic domain of Band 3, it does not account for the remarkable control of the RBC lifespan in the blood flow, and its relevance to aging or storage-associated senescence still has to be established.