

Comments on the paper “Synergistic effects of prostacyclin analogs and phosphodiesterase inhibitors on cyclic adenosine 3',5' monophosphate accumulation and adenosine 3'5' triphosphate release from human erythrocytes”

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Experimental Biology and Medicine 2014; 239: 385. DOI: 10.1177/1535370214522919

In the circulating blood, red cell ages vary from the youngest, the reticulocytes, which had left the bone marrow less than 24 h before, to the oldest whose age is about 120 days.^{1,2} Therefore, reticulocytes amount to about 0.8% of the circulating red cells. By staining all types of circulating blood cells using routine Wright staining or its variations, the youngest cells appear somewhat larger than mature red cells, and are polychromatophilic.³ The routinely employed specific staining method for reticulocytes uses New Methylene Blue.⁴ This stain demonstrates that within the reticulocyte population the cells vary in appearance. The youngest cells have a large amount of basophilic ribonucleoprotein, but have no nuclei from their preceding developmental stages within the bone marrow. Contrariwise, the most mature reticulocytes on the verge of becoming erythrocytes have but a single dot to identify their pre-erythrocytic state.^{5,6}

Knebel *et al.*,⁷ rather than washing circulating blood once in order to separate all of the erythrocytes from the other types of blood cells and plasma components, performed three centrifugations, each involving removal of its superficial red cells. The repeated centrifugations of blood followed by discarding the upper cells resulted in Knebel's group having analyzed not the normal circulating red cell mass whose cells vary as just described. Consequently, their findings may not be applicable to the circulating blood. The cells approaching their terminal stage as reticulocytes are the older ones of this group which despite having less ribonucleoprotein are heavier,^{8,9} presumably because they contain more fetal hemoglobin.⁸ Despite that, the lesser density of all reticulocytes results in the layering of these cells on centrifugation above the mature erythrocytes.⁹ Consequently, I suggest that for more physiological results

Knebel's group repeat its studies so that *circulating* blood cells, which are of all ages, are washed by centrifugation. A single centrifugation should suffice to layer the buffy coat of leukocytes and platelets above the red blood cells. This is followed by precise use of a syringe and blunt needle to aspirate the well-demarcated buffy coat which is then discarded, leaving red cells of all ages available for analysis. This would avoid biasing their findings toward the older cells.

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