

INVESTIGATION OF THE ORIGIN OF THE DENSITY HETEROGENEITY OF RABBIT PLATELETS. M.L. Rand, M.A. Packham and J.F. Mustard, Dept. of Biochem., University of Toronto, Toronto, Canada, and Dept. of Pathol., McMaster University, Hamilton, Canada.

Platelets are heterogeneous with respect to size, density and function. The question is unsettled whether platelets of different size and density are produced from megakaryocytes or whether large, heavy platelets become less dense as they circulate and are exposed to aggregating agents that decrease platelet density such as thrombin, plasmin, or ADP. Platelet subpopulations were separated by discontinuous Stratan density gradient centrifugation. Most dense platelets were larger than least dense platelets ($n=5$, $p<0.0025$). After labeling platelets *in vivo* with $^{35}\text{SO}_4^{2-}$, the incorporation and loss of ^{35}S in platelet density subpopulations was determined ($n=5$). At 1 hr, no ^{35}S was detected in the platelets. By 24 hr, ^{35}S was present in all density subpopulations but the relative specific radioactivity (RSR) of the most dense subpopulation was 7 times greater than that of the least dense, although the most dense platelets were only 1.2 times larger. The RSR of the most dense subpopulation reached a maximum between 48 and 72 hr and then decreased gradually whereas that of the least dense increased slowly, reaching its maximum at 96-120 hr. These results support the theory that platelets become less dense as they age. ^{51}Cr -labeled platelets were injected into rabbits and the loss of radioactivity in platelet density subpopulations was determined ($n=3$). The most dense subpopulation decreased in RSR more quickly than the least dense. The % radioactivity/% platelets of the least dense subpopulation increased from 0.58 to 0.82 while that of the most dense decreased from 1.46 to 1.13 over 72 hr. These observations could be due to either preferential removal of heavy platelets or decrease in density on aging. Although these results support the hypothesis that the majority of platelets released into the circulation are larger, more dense platelets which decrease in density on aging, platelets of all densities are released into the circulation. Thus the origin of platelet density heterogeneity cannot be ascribed solely to aging, although this does contribute to it.

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10:30 h

PRODUCTION OF THROMBOXANE B₂ BY PLATELETS IS RELATED TO THEIR DENSITY J.F. Martin⁺, T. Shaw⁺, J. Jakubowski⁺, D.G. Penington⁺ and T.J. Martin⁺, Melbourne University Departments of Medicine, St. Vincent's Hospital⁺ and Repatriation General Hospital⁺, Melbourne, Australia.

Platelets of different densities are probably produced from megakaryocytes of different ploidy. Thromboxane B₂ (TxB₂) production was studied in density dependent subfractions of the total platelet population.

Platelets were isolated by a new technique using velocity sedimentation into a polyvinylpyrrolidone-coated colloidal silica (Percoll) gradient followed by equilibrium centrifugation through a continuous linear Percoll gradient. Fractionation of the second gradient yielded density-dependent platelet subpopulations which were functionally competent and showed minimal leukocyte and plasma protein contamination. By pooling fractions and adding buffer, suspensions containing similar concentrations of platelets representative of low, intermediate and high density subpopulations were prepared, each containing 20-30% of the total population. Aliquots of each suspension were stirred in an aggregometer and thrombin or arachidonic acid (AA) were added to induce aggregation. After three minutes the reaction was arrested by addition of ice cold ethanol and TxB₂ measured by radioimmunoassay.

When 0.5 mM AA (final concentration) was the aggregating agent, for every 10⁸ platelet the least dense subpopulations produced 10.2 ng TxB₂, and the most dense subpopulations 22.4 ng TxB₂ (means of four experiments), whilst the intermediate density subpopulations gave intermediate values. However, thrombin at 0.5 or 1.0 units/ml produced similar amounts of TxB₂ in all subpopulations. In a second series of experiments, AA-stimulated serotonin secretion in density dependent platelet subpopulations correlated closely with platelet number.

These observations imply functional differences between platelet subpopulations.

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10:15 h

HUMAN PLATELET AGE CORRELATES WITH BUOYANT DENSITY, BUT NOT WITH SIZE. D. Mezzano, P. Catalano, K. Hwang, and R. H. Aster. Departments of Medicine and Pathology, Medical College of Wisconsin and Blood Center of Southeastern Wisconsin, Milwaukee, Wisconsin, U.S.A.

Following infusion of ^{51}Cr -labelled autologous platelets into normal subjects, high density (HD) and low density (LD) platelet cohorts were isolated by centrifugation in isosmotic arabino-galactan (Stractan). Specific radioactivity (SA) of LD platelets declined rapidly post-infusion ($T_{1/2} = 1.8$ days) but SA of HD platelets remained constant or increased over a 3-4 day period and gradually declined for 6-7 days thereafter. These differences were exaggerated when platelet cohorts enriched in LD or HD cells by slow centrifugation in high density albumin were labelled and transfused. Mean survival of a platelet cohort enriched with HD cells was significantly ($p < .02$) shorter (7.73 days) than that of a cohort enriched with LD cells (9.33 days). In normal subjects treated with aspirin, capacity for thromboxane synthesis was regained more rapidly ($p < .05$) in LD than in HD platelets. HD and LD platelets differed only slightly in mean volume (HD platelets = $7.57 \mu^3$, LD platelets = $6.87 \mu^3$, $0.05 < p < 0.01$).

These findings imply that under normal conditions in man, newly formed platelets are less dense on the average than total platelets and become more dense as they age in the circulation. Thus, SA of LD platelets declines rapidly as these platelets move into a more dense compartment and are replaced by newly formed, unlabelled platelets; SA of HD platelets remains constant or increases as labelled platelets enter this compartment in numbers equal to or greater than the number leaving it at the end of their life span. The similarity in mean volumes of LD and HD platelets suggests that platelet size is unrelated to platelet age under normal conditions.

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10:45 h

EFFECT OF AGE AND OPERATIONS ON PLATELET SEROTONIN (5HT) AND PLATELET VOLUME. R D Shuttleworth and J R O'Brien, St Marys Hospital, Portsmouth, England.

To pursue the concept that "activated" platelets (plats) *in vivo* might be deficient in 5HT (as well as being deficient in PF₄ - Abstract submitted), we collected citrated blood from 39 "healthy" controls aged 15-50 yrs and from 14 patients (25-75 yrs), 2-8 days after major surgery. The plat 5HT, the 5HT uptake and plasma 5-hydroxy-indoles (PPP 5HIs) were measured fluorometrically and the mean plat. volume (MPV) was measured using a Coulter S plus counter.

In health ($n = 39$) with increasing age the MPV (correlation: $r = 0.45$, $p < 0.01$), the plat 5HT ($r = 0.62$, $p < 0.001$) and 5HT uptake ($r = 0.45$, $p < 0.01$) all decreased while the PPP 5HIs increased ($r = 0.71$, $p < 0.001$). The plat 5HT was also positively correlated with 5HT uptake ($r = 0.42$, $p < 0.01$) and with MPV ($r = 0.45$, $p < 0.01$) and negatively with PPP 5HIs ($r = 0.43$, $p < 0.01$).

After operations ($n = 14$) irrespective of age the plat 5HT was lower than the controls' ($p < 0.001$) and the MPV and 5HT uptake were low, but not significantly so. The plat. count was significantly lower than the controls ($p < 0.001$). Again the plat 5HT correlated with MPV and 5HT uptake.

Differential centrifugation of normal PRP into platelet sub-populations with differing MPVs confirmed the correlation between MPV and plat 5HT.

It is concluded that both operations and increasing age are associated with the appearance of small relatively empty plats "exhausted" of 5HT which cannot take up 5HT. Have these plats been partially activated and undergone release with a subsequent decrease in volume? Alternatively, are large platelets relatively full of 5HT removed from the circulation, or not produced? (These findings support and extend the findings of Shuttleworth et al, Blood March 1981: in press).

The effect of age over 50 yrs has not yet been fully investigated.