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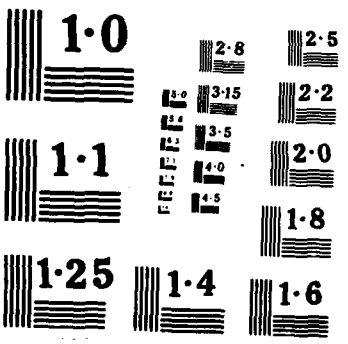
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SIXTH ANNUAL REPORT

HUMAN PLATELET SENESCENCE

ANNUAL SUMMARY REPORT

SIMON KARPATKIN, M.D.

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20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

1. Sequestration of megathrombocyte-enriched platelet populations by differential centrifugation in albumin, plasma or an arabino-galactan polymer; for purposes of platelet senescence studies as well as purposes of platelet transfusion and storage.
2. Preferential sequestration of megathrombocytes by the spleen.
3. Identification of microthrombocytes in disorders of increased platelet destruction.

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Annual Report for Human Platelet Senescence Study for 5/1/74 to 4/30/75

In the past year our laboratory has been engaged in 3 areas of platelet (and megathrombocyte) research with regard to production, turnover, identification, isolation and sequestration.

1. Sequestration of megathrombocyte-enriched platelet populations by differential centrifugation in albumin, plasma or an arabino-galactan polymer; for purposes of platelet senescence studies as well as purposes of platelet transfusion and storage.

2. Preferential sequestration of megathrombocytes by the spleen.

3. Identification of microthrombocytes in disorders of increased platelet destruction.

SUMMARY OF PROJECTS

1. Sequestration of megathrombocyte-enriched platelet populations by differential centrifugation in albumin, plasma or an arabino-galactan polymer; for purposes of platelet senescence studies as well as purposes of platelet transfusion and storage. Any information that would be helpful in developing better methods for platelet preservation or identification and preservation of young megathrombocytes would be of obvious help in the storage and administration of platelet concentrates. Since megathrombocytes (young platelets) have been shown to be more active functionally (1), as well as metabolically (2-4), isolation and storage of megathrombocytes would be more efficient than present systems of storage of entire platelet populations.

Two possible methods have recently been examined: Preliminary observations in humans as well as rabbits indicate that conventional methods for isolation of platelets via low speed centrifugation and removal of platelet-rich plasma provide total platelet yields of 65-85%, as determined from the initial whole blood volume and platelet count. We suspected that this loss of 15-35% of the platelet yield in the remaining white blood cell and red blood cell suspension might account for a significant number of megathrombocytes, since these have been shown to be heavier platelets. Preliminary studies, employing the P-64 channel analyzer attachment to the Coulter Counter, Model B, have revealed that this is indeed the case. Thus, after removal of the initial platelet-rich plasma and resuspending the remaining cell suspension in platelet-poor plasma, one can isolate an enriched population of megathrombocytes (as determined by the platelet volume distribution curve) representing approximately 10% of the total platelet yield. These observations are supported by other studies with human platelet-rich plasma when it was repeatedly recentrifuged at increasing RPM. The remaining platelet-rich plasma, after each centrifugation, was examined for its platelet-volume distribution curve. A graded shift of the curve towards the left (smaller platelets) was obtained with increasing RPM, indicating that heavier-larger platelets were removed earlier, at lower RPM (see figure 1).

Preliminary observations in humans, indicate that megathrombocyte-enriched platelet populations can be isolated from whole blood on a stractan (arabino-galactan polymer) density gradient. This material has been studied for its use as a density gradient suspending media for the separation of red blood

cells (5). It has advantages over previously employed oil density, and albumin density gradients in that:

1) It is inexpensive and can be used for large scale preparations.
2) It is relatively inert osmotically as opposed to albumin.
3) It is not deliquescent as opposed to albumin.
4) It is not contaminated with salts, as opposed to albumin.
5) It is soluble in aqueous media as opposed to oil density gradients.

Preliminary studies employing platelet volume distribution curves of platelet populations isolated from whole blood placed on this gradient reveal a better than 95% total platelet recovery; and confirm previous observations that large platelets are heavy platelets. These isolated platelet populations are easily suspended in platelet-poor plasma.

2. Preferential Sequestration of Megathrombocytes by the Spleen. We have recently made the interesting observation that the spleen preferentially sequesters megathrombocytes (6,7) (see enclosed manuscript). This was established via 4 different approaches: 1) Splenic blockade with phenylhydrazine, which revealed an apparent splenic platelet pool of 40% compared to a splenic megathrombocyte pool of 52% 2) Rabbits studied post-splenectomy, which revealed an apparent splenic platelet pool of 30% compared to a splenic megathrombocyte pool of 55% 3) Epinephrine injection into rabbits as well as dogs, which revealed an increase in platelet count of 1.3 fold compared to an increase in megathrombocyte number of 3 fold, with peak response in 2 to 6 minutes. Platelet volume distribution curves which revealed a marked shift to the right, indicating the release of large platelets, following epinephrine injection 4) Splenic massage of surgically exposed spleens which revealed a rise in megathrombocyte number of 1.6 fold in the absence of a rise in platelet count. It is postulated that the spleen is utilized as an emergency store of megathrombocytes (enhanced platelet-function and metabolic potential), which can be mobilized in time of stress, i.e. epinephrine.

3. Identification of Microthrombocytes as well as Megathrombocytes in Disorders of Increased Platelet Destruction. The Coulter Counter, Model B, has been a useful instrument for the detection of large platelets, megathrombocytes, on the right side of the platelet volume distribution curve. With the availability of the P04 Channel Analyzer attachement, the left side of the platelet volume distribution curve (small platelets) could be more reliably studied, i.e. less electronic noise, and greater sensitivity. Focusing on this part of the curve, we have made the interesting observation of increased microthrombocytes during situations of increased destruction or utilization of platelets (8). The microthrombocytes are apparent as either a separate peak on the left side of the curve, or a shift of the curve to the left. This is associated with the usual relative increase in megathrombocytes previously reported. Patients with autoimmune thrombocytopenic purpura, as well as other patients, have this newly-identified platelet volume distribution pattern. These curves have also been reproduced in rabbits following the injection of anti-platelet antibody. Electron microscopy of blood from patients with microthrombocyte peaks reveal very small intact platelets as well as platelet fragments (see enclosed manuscript).

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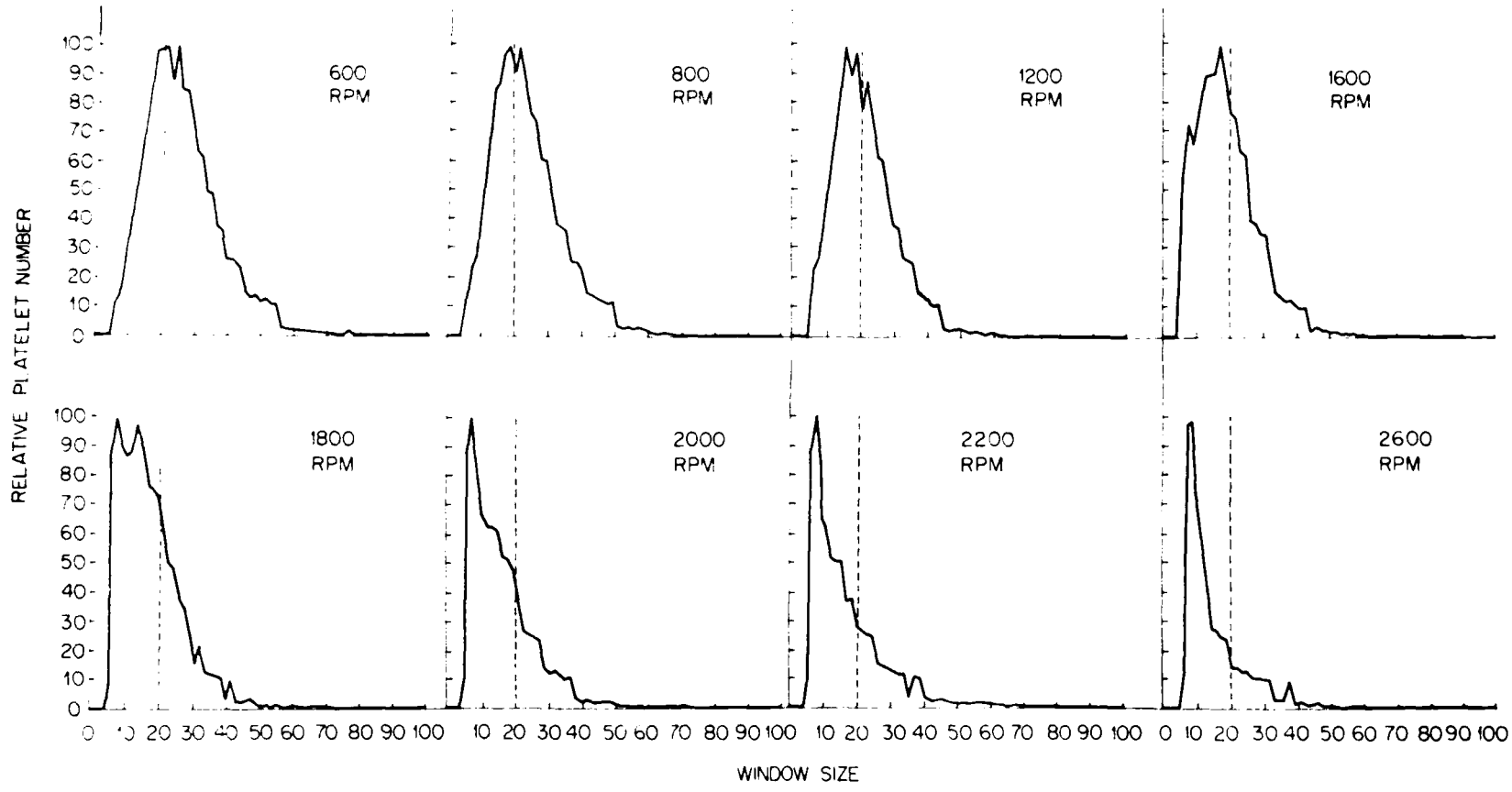
600 RPM
800 RPM
1200 RPM
1600 RPM
1800 RPM
2000 RPM
2200 RPM
2600 RPM
RELATIVE PLATELET NUMBER
WINDOW SIZE

FIGURE 1

END

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