Thrombocytogenesis in Surgical Patients

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Comparative studies of peripheral blood and bone marrow have shown that the postoperative marked and sustained reduction of blood platelets is accompanied by a block in the mechanism of delivery of platelets into the blood stream and a delay in maturation of blast cells of the megakaryocyte lineage.

A CARDINAL feature of more recent contributions to our understanding of the physiologic responses to trauma has been a growing realization of the unique importance of platelets in the mechanisms of hemostasis. If, as has been suggested, platelet alterations are the major determinant in the pathogenesis of postoperative thrombophilia, information concerning the mediation of the responses which have been observed may provide a rational approach to the prevention of thromboembolism.

A consistent pattern is discernible in the fluctuations of circulating platelet concentration following major surgery. A fall in concentration of platelets early in the course of a major operation terminates in a thrombocytopenia which is sustained until the third or fourth postoperative day. The ensuing upward trend usually returns the platelet count to its preoperative range by the seventh day and, in a majority of patients, "overshoots" to a level of relative thrombocytosis during the latter part of the second week of convalescence.

While it appears that augmented megakaryocyte productivity, as interpreted from observations previously reported from this laboratory, may be responsible for the "high tide" of the platelet response, evidence that marrow function is a significant factor during the "low tide" phase has been inconclusive. Although observations that the average survival time of circulating platelets is at least four days discredit the significance of production and delivery processes in the rapid fall in peripheral platelet counts during operation, a study of megakaryocyte morphology during this changing period may elucidate the mechanism by which the thrombocytopenia is produced and sustained.

The present report deals with the salient features of our renewed investigations, focused on clarification of previously observed discrepancies in thrombocytogenesis during the early postoperative interval.

Materials and Methods

This report is based on parallel observations of the bone marrow megakaryocyte morphology and of peripheral blood platelet concentration in 15 hematologically normal adult male patients before, at the conclusion of, and on the second day following elective major surgical operations; none of the subjects had evidence of hemorrhage pre- or postoperatively; all convalesced uneventfully and without thromboembolic sequelae.

Additional platelet counts were done on the first and third postoperative days to assure that the time of the final marrow sampling in each patient did, in fact, lie in the interval of low ebb of the platelet tide.

The marrow smears which formed the basis for the report of Kerhulas, et al. were reviewed as an adjunct to the present investigation. In the four instances in which the clinical criteria met the standards of the current inquiry, our revised appraisal of the megakaryocyte differentials is incorporated in the observations from 11 patients more recently studied.

Platelet Counts.—Platelets were counted by the direct method, using Pohle's modification of Rees-Ecker diluting fluid, on antecubital venipuncture specimens with dried balanced double oxalates as anticoagulant in accordance with the technique previously detailed. All determinations were made by one investigator experienced in platelet counting.

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with a coefficient of variation for a single count not greater than 10 per cent.  

**Bone marrow biopsies.**—Bone marrow smears were made on glass slides from 0.2-0.3 cc. of marrow (without anticoagulants) obtained by needle aspiration biopsy of vertebral spinous processes in the upper lumbar or lower thoracic region. The air-dried preparations were then treated in succession with Wright's and Giemsa's stains.

Fine lines drawn with India ink across the under surface of the stained and mounted slides were used to simplify "high dry" and oil immersion microscopy of consecutive fields for the 200 cells catalogued for each differential.

**Megakaryocyte classification.**—For description of megakaryocyte morphology we have employed the two-parameter scheme of De La Fuente. This method of tabulation takes cognizance of the fact that the correlation between platelet formation and morphologic maturity is evidently of low degree, and permits characterization of a cell of the thrombocytopoietic series both as to its station in the generally accepted maturation sequence (megakaryoblast → promegakaryocyte → megakaryocyte) and as to the number of discrete platelet units it contains.

The histologic features which distinguish the cell types within the megakaryocyte maturation profile have been presented by Dameshek and Miller. Except for use of the minor modifications of nomenclature employed by De La Fuente these criteria were followed.

In deciding upon a numerical (or alphabetic) designation for the second or "productivity" parameter of a cell we have, as in the earlier study, followed De La Fuente in counting only those intracellular platelets that are peripherally located although we can no longer subscribe to De La Fuente's conviction, predicated on the plausibility of regarding platelets so situated as "available" to the marrow circulation, that this parameter directly measures the contribution of a megakaryocyte to the circulating platelet mass at the instant of marrow biopsy.

According to its platelet content each cell is assigned to one of four alphabetically represented groups: "A" indicates that platelets, if present at all, are centrally located in the cytoplasm of the cell; "B" denotes a content of one to ten; and "C" more than ten platelets; "D" describes cells whose only extra-nuclear substance is an aggregate of mature and individually distinct platelets.

In the absence of any direct evidence that dispersion of platelets is an exclusive franchise of cells of full morphologic maturity, we have treated the two differential parameters as independent variables except in the case of the "D" form, whose nuclear characteristics have invariably been those of fully matured megakaryocytes.

We have recognized the "D" form in a wide latitude of sizes and shapes (fig. 1) with certain features in common: 1. The smaller variants may escape notice if only a low magnification survey of the smear is made. 2. The platelets in the aggregate are individually distinct, do not appear "fused" or "clumped". 3. The cell membrane, when distinguishable at all, is usually disrupted. 4. The nucleus (whose presence, of course, must be unequivocal) has frequently undergone pyknosis and karyorrhexis.

"D" cells have been a prominent feature in our observations. We have exercised caution in their recognition to avoid deception by artefacts of superimposition (i.e. the fortuitous association of agglutinated platelets)
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Tinuted platelets from marrow blood with fragments of nuclei broken by smearing). However, as others have remarked,13 in a thrombocytopenic state (the circumstance prevailing postoperatively) such fallacious appearances cannot introduce significant error because of the paucity of circulating platelets.

OBSERVATIONS

The data are presented in composite forms in tables 1 and 2 and in figure 2. Since, in every patient, the changes observed were of the same sign and of similar amplitude, the "mean" values given typify individual as well as group characteristics.

Changes in Peripheral Blood Platelet Counts.—During operation (a period of approximately four hours), as illustrated in figure 2, the concentration of circulating platelets fell to less than half its preoperative mean, initiating a thrombocytopenia which changed little in the ensuing three days.

Changes in Megakaryocytes.—No attempt was made to estimate relative or absolute numbers of megakaryocytes in these serial marrow aspirations since available methods for quantitation of these marrow elements are of doubtful value in the absence of an absolute frame of reference.

Evidence of Altered Productivity.—Although the percentile total of "B" + "C" + "D" forms (all of the platelet containing cells) remains unchanged following operation, the superficial nature of this apparent placidity becomes evident when this estimate of overall productivity is factored into its cardinal components (by segregation of "B" + "C" from "D" forms). As figure 2 illustrates, the total percentage of "B" + "C" forms appears as a close parallel of the circulating platelet count while the percentage of "D" forms appears as a paradoxical platelet-rich
appearance at a time when the peripheral blood is platelet-poor.

This state of altered equilibrium appears unchanged in the marrow picture of the second postoperative day, accompanied by the continued thrombocytopenia.

Evidences of Altered Megakaryocyte Maturation.—The changes in maturation dispersion are recorded in table 1 and table 2. A marked "shift to the left" results in a more than twofold increase in the ratio

$$ \frac{\text{megakaryoblasts ("A" + "B" + "C")}}{\text{promegakaryocytes ("A" + "B" + "C")}} $$

There appears to be also a "shift to the right," reducing the ratio

$$ \frac{\text{promegakaryocytes ("A" + "B" + "C")}}{\text{megakaryocytes ("A" + "B" + "C" + "D")}} $$

from 0.57 to 0.21, a reversal resulting solely from an increase in the "D" component of the denominator of this ratio from 5.6% to 51.2%.

This pattern is again evident on the second postoperative day.

Correlations and Interpretations

The promptness of the appearance of alterations of megakaryocyte morphology is not, in principle, an unprecedented observation. Utilizing the techniques of phase microscopy in examining living and unstained marrow specimens, Pisciotta et al., found that cytologic changes in megakaryocytes were recognizable thirty minutes, and were far advanced two hours following induction of a temporary thrombocytopenia in normal individuals by infusion of plasma with antiplatelet agglutinins of high titer.

Peripheral Platelet Levels and Megakaryocyte Productivity Patterns.—An assumption that a megakaryocyte's content of "available" platelets is a measure of its actual platelet output, or that the percentile representation of platelet-containing cells within the megakaryocyte productivity dispersion varies directly as the concentration of platelets in peripheral blood is not compatible with our data. On the contrary, we have observed an inverse correlation between percentage of cellular reservoirs of "available" platelets (i.e. "D" forms) in the marrow and the circulating platelet level (fig. 2).

We have interpreted the persistence of this inverse relationship during the early postoperative interval as indicating that these levels represent the circumstances of a new state of equilibrium, rather than a continuing negative platelet balance. If this is a valid inference (in part, corroborated by the lack of evidence that postoperative platelet expenditures can account for the observed deficit), the abrupt rise in per cent "D" forms takes on an additional significance: if it is neither cause nor direct result of the state of thrombocytopenia, the two phenomena (increase in "D" per cent and decrease in circulating platelet count) may be interconnected by a common causation. In terms of the marrow response, this means a block in the mechanism of dissociation of platelets from megakaryocytes into the peripheral circulation.

These perspectives help resolve the enigmatic increases in megakaryocytes at both extremes of the productivity dispersion. The percentage of "D" forms may be regarded as an inventory of finished but as yet unmarketed products and not as a measure of active producing at the instant of marrow biopsy. Exclusion of the "D" forms from the productivity index brings out the twofold increase on the ratio $A\% / (B\% + C\%)$, suggesting that the formation of new platelets is being retarded.

Megakaryocyte Maturation Patterns.—The foregoing considerations serve also to circumvent the contradiction of "shifts to left and right" implicit in observed derangements of the maturation profile. The rise in % "D" forms, responsible for the "shift to the right," from our interpretation reflects merely the inability of the marrow to rid itself of the end results of past activity and signifies neither increased platelet producing nor hastening of cellular maturation.

It seems likely that the twofold increase of the ratio $\frac{\text{megakaryoblasts (A + B + C)}}{\text{promegakaryocytes (A + B + C)}}$ is also a product of the same retarding influence. It is of great interest that a similar shift has
been observed in other conditions, notably idiopathic thrombocytopenic purpura (14, 10, 9).

Cellular development beyond the 'blast stage is apparently unaffected (ratio of promegakaryocytes (A + B + C) to megakaryocytes (A + B + C) remains constant.)

**SUMMARY**

Parallel observations were made of the bone marrow megakaryocyte morphology and of peripheral blood platelet concentration in 15 hematologically normal adult male patients before, at the conclusion of, and on the second day following elective major surgical operations.

The changes in the concentration of circulating blood platelets uniformly consisted of a fall to a level which averaged 45 per cent of the preoperative mean. This thrombocytopenic state persisted for the remainder of the period of observation.

The concomitant alterations in the observed morphological characteristics of the bone marrow megakaryocytic series were similarly unvarying and consisted of prompt and sustained increases in the relative numbers of cells at each extreme of both the maturation and the "platelet production" dispersions. The magnitude of these changes is indicated by a more than twofold increase in the ratio of megakaryoblasts to promegakaryocytes, and a tenfold increase in the percentage of "post-mature" platelet-laden megakaryocytes.

The observed patterns of response have been interpreted as signifying that a major surgical operation results in a suppression of platelet formation within megakaryocytes, a block in the mechanism of "delivery" of platelets from the marrow into the blood stream, and a hindrance to maturation of 'blast cells of the megakaryocyte lineage, but no derangement of maturation of more mature elements.

A common agency may be responsible for the restriction of platelet deliveries, the reduction of circulating platelets to thrombocytopenic levels, and the apparent hindrance to cellular maturation.

**REFERENCES**

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