Platelet Size in Thrombocytopenias and Thrombocytosis of Various Origin

By M. Kraytman

Planimetric studies were carried out on canine blood platelets fixed with formaldehyde. In the normal dog, the mean platelet area was 5.25 sqμ (SEM ± 0.34 sqμ). During the first 3 days following acute experimental thrombocytopenia, platelet area increased, averaging 9.5 sqμ (p < 0.01). The reactive thrombocytosis following splenectomy or nephrectomy was not accompanied by a rise in large platelets. Platelet size decreased to 3.9 sqμ in dogs made thrombocytopenic by intravenous injections of mitomycin C. The morphologic changes induced by the various experimental procedures suggest that: (1) newly formed platelets are larger than platelets of a normal population; (2) some large platelets are possibly released by macro-megakaryocytes; (3) other large thrombocytes probably represent fragments of granular megakaryocytes; (4) senescence of platelets in the circulation is associated with decreasing size; and (5) large young platelets are preferentially retained in vivo on glass beads.

Several recent studies have shown that newly formed platelets are larger than older ones. However, not all authors agree with the view that the size of platelets diminishes during their survival in the peripheral circulation. Enticknap et al. claimed that senescence of platelets, unlike in many other cytotoxic lines, was associated with increasing size. Mannucci and Sharp, investigating the relationship between platelet size and function, stated that "it is still not certain whether young platelets are large or small." Minter and Ingram observed that not all new platelets were large and that frequency distribution histograms of platelet volumes were markedly changed only in the case of severe platelet depletion. Physical, metabolic, as well as functional, evidence suggest a relationship between platelet heterogeneity and platelet age. Therefore, accurate information about platelet size as related to platelet age seems important, especially as Garg et al. have recently reported that the large platelets could also be used as an index of megakaryocyte proliferation.

In recent studies, platelet size has generally been expressed as volume determinations. Detwiler et al. measured relative volumes of young, old, and normal packed platelets in microhematocrit tubes. Other authors determined platelet size with electronic particle counters coupled with a particle size distribution plotter. However, these methods are subjected to potential sources of error, which have been reviewed elsewhere. Bigel et al. and Garg et al. measured the diameter of platelets by means of a micro-

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metric ocular. As the state of medullary stimulation influences the shape of platelets, a precise definition of their diameter might be difficult. Planimetry of platelets appears to be an interesting method, since it avoids the problem associated with diameter determination. However, there are only a few reliable data relative to platelet area estimation. We decided, therefore, to investigate systematically, in the dog, the surface and morphology of platelets in normal thrombocytopoiesis and thrombocytosis or thrombopenia of various origins, in order to provide additional quantitative data about platelet production.

MATERIALS AND METHODS

Mongrel dogs of both sexes, weighing between 14 and 22 kg, were used. They were divided into six experimental groups.

Group 1
Twenty normal dogs were studied as controls before further experimentation.

Group 2
Six dogs were subjected to an acute experimental thrombocytopenia. This method, described elsewhere, utilizes the continuous extracorporeal circulation of blood through a column containing glass beads, 1 mm in diameter, to which the platelets adhere. The platelets decrease to about 5% of the initial count at the completion of the procedure that lasts 3 hr.

Group 3
In five dogs, a reactive thrombocytosis was induced by splenectomy under Nembutal anesthesia. The operation lasted on the average 30 min.

Group 4
In five dogs, a postoperative thrombocytosis was induced by left nephrectomy under Nembutal anesthesia. This operation was considered equivalent to a splenectomy insofar as magnitude and duration of the surgical stress.

Group 5
Four dogs were treated with mitomycin C, in order to assess the effect of inhibition of new platelet production on the platelet size. The animals received a total of four intravenous injections of mitomycin C (0.2 mg/kg every 2 days).

Group 6
Four dogs were subjected to acute thrombocytopenia 2–5 mo after splenectomy to eliminate the possibility that newly formed platelets were sequestered in the spleen. Blood was drawn from the femoral artery. One milliliter of blood was mixed with 1 ml of citrate-formaldehyde mixture. Thin blood films were stained with May-Grünewald-Giemsa stain. This formalin fixation avoids the clumping of platelets so frequently observed on dry smears of unfixed blood, particularly in the case of thrombocytosis. Furthermore, it has been demonstrated that the platelets and erythrocytes maintain a homogenous distribution during the whole procedure. Microscopic fields were examined under oil immersion in a zigzag fashion. Platelets were drawn, using a camera lucida, at a magnification of 1000 times. The drawings obtained were magnified five times, and planimetry was performed with an A-Ott apparatus (Kempten-Bayern). The area of 200 platelets in each dog was measured in triplicate. The mean of these determinations was translated from planimetric units to platelet area in sq. μ by applying an appropriate conversion factor. The planimeter was calibrated by measuring the circumference of varying known
areas. Reproducibility of the measurements was at least 90%. The shape of the platelets, as well as the appearance of their cytoplasm, was noted. Platelet counts were made on arterial blood in duplicate by the method of Piette and Piette with a phase microscope using 200 times magnification. Microhematocrit was also determined on arterial blood.

These determinations were made before any experimental procedure in the controls (group 1); immediately after the experimental thrombocytopenia (groups 2 and 6); every day during 8 days after experimental depletion (groups 2 and 6), splenectomy (group 3), or nephrectomy (group 4); and on the day the platelet count was less than 10% of the initial value in the mitomycin C-treated dogs (group 5).

RESULTS

Platelet area distribution curves in all experimental groups were markedly asymmetrical. Approximate normalization of these curves could be achieved by plotting the cumulative frequency of size distribution on a gaussian probability scale against the logarithms of the area values. Logarithmic values were used for statistical comparisons.

Group 1

In the control group, the mean platelet count was 184,000/cu mm (SEM ± 5800). The platelet surface area distribution is recorded on Fig. 1. The mean platelet surface area was 5.25 sq μ (SEM ± 0.34 sq μ). The term “large platelet” was restricted to thrombocytes as large as or larger than twice the mean area value. Their mean value was 500/cu mm (SEM ± 1200/cu mm)

Fig. 1. Platelet size distributions in normal dogs, at the completion of the acute experimental thrombocytopenia (hour 3), and from 1 to 8 days after this procedure.
or 4.6% of the circulating platelets. Platelets equal to or larger than three times the mean value amounted to 0.5%. At the left extremity of the curve, 5% of the platelets had area values smaller than the half of the mean value. Thus, the area of approximately 90% of the platelets varied between $2.6 \text{ sq } \mu$ and $10.5 \text{ sq } \mu$. The great majority of platelets were round, sometimes oval, with a sharply defined and smooth outline. In most platelets, the granules were evenly distributed throughout the cell. Other thrombocytes distinctly showed a hyalomere surrounding a granulomere. Platelets were well separated. Sometimes, two or even three thrombocytes were lying close together, but clumping was never observed. No pseudopod formation was noticed. (Fig. 2A).

Large platelets constituted a heterogeneous population, of which approximately 50% (2.4% of the planimetered platelets) showed very irregular shapes—more or less rectangular, horseshoe- or sausage-shaped, elongated, or totally irregular. Their cytoplasm differed strikingly from the cytoplasm of the other "large" platelets. It was uniformly filled by numerous, deeply stained granules (Fig. 2B). These platelets were called "giant" thrombocytes. To the other
large platelets, characterized by a round smooth shape and presenting a
granulomere and hyalomere, the term “megathrombocyte” was applied. (Fig.
2C). Platelets smaller than 2.6 sq μ were generally deeply stained and had a
compact appearance.

**Group 2**

At the completion of the acute experimental thrombocytopenia, the mean
platelet count dropped to 9200/cu mm (SEM ± 400/cu mm). The mean area
value was 3.34 sq μ (Fig. 3). This value is significantly different from the normal
mean value in controls (p < 0.01). The platelets had generally a rounded shape
and a dense aspect, lacking the differentiation into hyalomere and granulo-
mere. Aggregates, composed of five or ten or more platelets, were occasionally
seen. Isolated, as well as clumped, platelets showed nearly always fine small
dendritic pseudopodia (Fig. 2D). Megathrombocytes, as defined above,
amounted to approximately 100/cu mm (0.5%), and giant thrombocytes were
no longer visible.

After the acute depletion, the peripheral platelet counts rose slowly until
day 3–4, then increased more rapidly to overshoot the normal range by day 5.
and remained still higher than the initial count on the eighth day (Fig. 3). The
mean values of platelet area on days 1, 2, and 3 averaged 9.5 sq μ (Fig. 3),
differing significantly from the controls (5.25 sq μ, p < 0.01), and then declined
toward the initial value that was reached on day 7. The absolute values of large
platelets (≥ 2 × 5.25 sq μ) on the days following the acute depletion are
recorded on Fig. 4. Starting from 100/cu mm at the end of the experimental
procedure (3-hr determination), they increased to 5000/cu mm (37.6%) about
20 hr later, amounted to 11,000/cu mm (42.5%) on day, 2 and reached
an absolute peak on day 4 (25,800 large platelets/cu mm), the megathrombocytes being more numerous than the giant thrombocytes (Fig. 4A). Approximately 90% of the megathrombocytes had areas of two to three times the control value, whereas the majority of the giant thrombocytes was at least three times as large as normal platelets (Fig. 4). Megathrombocytes returned toward their basal value on day 8, i.e., 3 days later than giant thrombocytes.

About 2.5% of the platelets, mostly large ones, presented interesting morphologic features on days 1–5. Some showed an indentation that gave them a peanut form or bilobed appearance; others had a dumbbell form. In this case, both rounded extremities contained granules in their center with an outer zone of hyaline cytoplasm. The two rounded appendages were connected by a stretched-out cytoplasmic strand of variable length and width (Fig. 2E). Rarely, large platelets showed three “lobes” joined by a thin thread of cytoplasm (Fig. 2E). The area of the round lobes was generally smaller than 5 sq μ.

Group 3

After splenectomy, circulating platelets decreased slightly on day 2, and then overshot the initial value on day 3. They reached higher levels than after an acute experimental depletion (Fig. 3). The mean platelet area did not vary significantly (Fig. 3). The megathrombocytes and giant thrombocytes likewise remained at their basal values.

Group 4

After a nephrectomy, the peripheral platelet counts oscillated around the
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initial value during the first 4 days. They began to rise significantly on day 5, without reaching the levels observed after an experimental thrombocytopenia (Fig. 3). The mean area of platelets, as well as the proportion of large thrombocytes, showed nonsignificant variations (Fig. 3).

Group 5

In dogs injected with mitomycin C, platelets dropped to less than 10% of their initial value between the fourth and seventh day after the last intravenous injection. The mean area value of platelets, planimetered on the day when this severe thrombocytopenia occurred, was significantly decreased (3.9 sq μ; p < 0.05).

Group 6

The reappearance of circulating thrombocytes after acute platelet depletion in previously splenectomized dogs was, despite the high initial mean value (364,000/cu mm), similar to the pattern of response observed in intact animals (group 2). Likewise, variations in size distribution and percentage of megathrombocytes and of giant thrombocytes were identical in both groups.

DISCUSSION

In this study, the mean area of normal canine platelets averaged 5.25 sq μ. Assuming that platelets have a spherical form, this value corresponds approximately to a diameter of 2.6 μ and to a volume of 9.2 cu μ. Minter and Ingram,7 using an electronic particle size analyzer, found values in the dog between 7.67 and 8.99 cu μ. Nakeff and Ingram,16 utilizing the same method, reported a value of 7.17 cu μ for canine platelet volume. These discrepancies are probably due to experimental factors, such as temperature, surrounding medium, and anticoagulants.17 Platelets with an area as large as or larger than twice the mean control value were herein defined as “large” platelets. They amounted to approximately 5% (8500/cu mm) in control dogs. During the platelet depletion, these large platelets were preferentially removed, as shown by the shift to the left (small thrombocytes) of the platelet size distribution at the end of the procedure (Fig. 1, 3-hr determination). At this time, large platelets amounted to 100/cu mm. About 20 hr later, when circulating platelets averaged 13,400/cu mm, the large platelets increased to 5000/cu mm and remained increased in absolute numbers from day 3 to day 7, and in proportion from day 1 to day 5 (Fig. 4). The fact that we observed identical increases of large platelets in normal and asplenic dogs after severe acute platelet depletion seems to eliminate splenic sequestration of newly formed platelets in this species.13 On the other hand, the relative increase of large platelets did not occur after splenectomy or a surgical stress such as nephrectomy and, thus, is not necessarily related to reactive thrombocytosis itself. As the relative and absolute increase of large platelets occurred concomitantly with a period of platelet depletion, it could be explained by an increased peripheral destruction involving preferentially the smaller platelets. This mechanism is unlikely, since previous studies excluded intravascular coagulation with platelet consumption during the period of relative thrombocytopenia.25 Furthermore, Ebbe et al.26
have shown in the rat that newly formed platelets were destroyed at a normal rate when transfused into normal recipients. Consequently, it seems more likely that the increase of circulating large platelets reflects the medullary stimulation induced by an acute peripheral thrombocytopenia.

However, we observed that in control, as well as in platelet-depleted, animals these large platelets formed a mixed population, in which two main types of cells could be distinguished, the “megathrombocytes” and “giant thrombocytes.” These cells differed by size, shape, and cytoplasmic aspect, as well as by their response pattern to acute platelet depletion. It is of interest to consider the possible mechanisms underlying these morphologic and functional differences.

Previous studies have shown that acute thrombocytopenia produces an increase in megakaryocyte number, size, and maturation rate. Our data suggest that megathrombocytes, as defined above, are young, newly formed platelets probably produced by the macromegakaryocytes. Several experimental results are in agreement with this hypothesis. Ebbe et al. showed that enlargement of stage III megakaryocytes, induced by thrombocytopenia, persisted for 3–5 days, depending on the severity and duration of the platelet depletion. This observation could be correlated with the rise in number of the megathrombocytes, which remained increased above basal values for about 7 days following experimental thrombocytopenia (Fig. 4). It has also been shown that surgery induced a reactive thrombocytosis with an acceleration of the megakaryocyte maturation, without macromegakaryocytosis. Since we did not observe megathrombocytosis in dogs subjected to splenectomy or nephrectomy, it seems that macromegakaryocytosis and increase in circulating megathrombocytes are intimately related.

Giant thrombocytes had irregular shapes. Their cytoplasm contained numerous granules like the cytoplasm of the granular megakaryocyte. The morphologic features of the giant thrombocytes strongly suggest that these cells should probably be considered as megakaryocytic fragments rather than as large thrombocytes.

It has been demonstrated in rats that acute thrombocytopenia was followed by a delay of about 2 days before increased production of platelets occurred, suggesting that the stimulus arising from thrombocytopenia affected precursor cells. In the dog, the maturation time of the radioresistant compartment of megakaryocytes appears to be about 4 days, as suggested by radiation data of Cronkite et al. and Kagnoff. In the present study, after acute platelet depletion, large platelets were increased in absolute numbers above basal values on day 3 (Fig. 4A). Therefore, one could propose that, in the thrombocytopenic animals, the normal megakaryocytic maturation time was shortened to about 3 days in response to the thrombocytopenia. The increased proportions of large platelets on the first 2 post-thrombocytopenic days can not be equated with an increase in platelet production, since with experimental depletion of the circulating platelet population to about 5% of normal, the production of a normal number of new large platelets would result in an increased proportion of large platelets in the circulation simply because the number of smaller platelets has been reduced. However, large
platelets with an area of at least three times normal, mostly giant thrombo-
cytes, were increased at 2 days (Fig. 4B), suggesting that the thrombocytopenic
stimulus could exert its effects not only on the megakaryocytic precursor cells,
but also on the recognizable granular megakaryocyte, at a level preceding
normal maturation. An acute peripheral thrombocytopenia could possibly
stimulate these cells to release into the circulation more fragments of their
cytoplasm than in basal conditions. A somewhat similar model for the
regulation of granulopoiesis has recently been proposed. Morley and
Stohlman33 have postulated that neutrophil delivery to the blood is controlled
by two feedback loops: one controlling bone marrow cell production and the
other acting on cell release and permitting rapid increases of blood granu-
locytes. By analogy, our data could be compatible with the following sequence
of events: acute thrombocytopenia elicits a rapid and increased release of
fragments from granular megakaryocytes before completion of maturation.
This mechanism induces an immediate, relatively short-lived, minor increase
of blood platelet concentrations. Meanwhile, the thrombocytopenic stimulus
induces the formation of macromegakaryocytes, producing “megathrombo-
cytes” after a delay of about 3 days. Acute thrombocytopenia also accelerates
stem cell differentiation. This could explain the lag period of 3 days observed
in the reactive thrombocytosis, corresponding to the time needed for the
precursor cell to become a mature megakaryocyte releasing normal-sized and
-normal shaped platelets. Accordingly, the reactive thrombocytosis that follows
nephrectomy, after a lag period of approximately 4 days, might also result
from stimulation at the level of committed precursors.34-35 The highly reactive
thrombocytosis observed after splenectomy could be mediated by the same
feedback mechanism evoked by any surgical procedure and accentuated by
the removal of the splenic trap. Postsplenectomy thrombocytosis was slightly
delayed, in spite of the removal of a site of platelet sequestration. This has
been reported elsewhere36 and has been attributed to an eventual consumption
of platelets at the site of the operation.37 We did not observe a rise in large
thrombocytes after splenectomy or nephrectomy. Garg et al.15 reported an
increased percentage or number of large platelets during conditions of
increased destruction, as well as increased production of thrombocytes. This
observation concerned patients with various chronic hematologic disorders,
whereas we studied acute experimental thrombocyte modifications in the dog.
It should also be emphasized that, in the terminology of these authors, a
megathrombocyte had a diameter of more than 2.5 μ, whereas a large throm-
boocyte, as herein defined, had a diameter of about 3.6 μ or more. This stresses
the need for a uniformly adopted definition of large platelets.

In the dogs subjected to acute thrombocytopenia, the mean platelet area
decreased, beginning on day 4, at a time when the platelet count was still rising
(Fig. 3). Ebbe and Phalen38 observed a similar discrepancy in rats during
recovery from thrombocytopenia. This decrease in platelet size could be
attributed to production of smaller platelets by megakaryocytes that had by
then returned to a normal size, as well as to aging of the platelets in the
circulation. Our findings in mitomycin C-treated dogs are in agreement with
the hypothesis that some large platelets become smaller as they age in the
circulation. In these animals, a decrease in the mean area of the remaining blood platelets was concomitant to thrombocytopenia. One could speculate that the cytoxic agent selectively acted on macromegakaryocytes to inhibit production of large platelets in the marrow. To our knowledge, such a preferential inhibition of large cells has hitherto not been reported. A more likely interpretation would be that mitomycin C inhibited new platelet production and that the size of platelets released into the circulation before medullary inhibition decreased as they aged. Accordingly, Spertzel et al.\textsuperscript{39} reported in dogs a decrease in number of larger platelets in the first week following total body irradiation. However, data of Minter and Ingram\textsuperscript{7} suggested that large dense platelets produced following acute blood loss did not decrease in volume as they aged. On the other hand, our morphologic observations suggest, but do not prove, that some large platelets could release two small platelets with maturation (Fig. 2E). Zucker-Franklin\textsuperscript{44} has recently pointed out that biochemical data seem to support the concept that circulating large megakaryocyte fragments could break down into smaller forms during their lifespan in the circulation. However, definite demonstration of this postulated phenomenon could only be made by microcinematography. The transformation of giant thrombocytes into megathrombocytes seems unlikely as we did not observe a sustained rise in circulating megathrombocytes after the peak reached by the giant thrombocytes on day 4 following the acute depletion.

The platelets remaining in the circulation immediately after the procedure of experimental thrombocytopenia were significantly smaller than platelets of a normal control population and showed nearly always small pseudopods. Hovig and Hellem\textsuperscript{41} reported the same phenomenon of extensive pseudopod formation in platelets after passage through a column of glass beads. It seems probable that the larger (and younger) platelets were preferentially retained on the glass beads and that the emerging small “activated” spiny thrombocytes were the older ones. Conflicting results have been reported about platelet adhesiveness to glass as related to size.\textsuperscript{6,13,14,42} Our data are in agreement with studies showing that large young platelets are more adhesive to glass than older platelets.\textsuperscript{13,14}

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