Alterations in Numbers of Circulating Platelets Following Surgical Operation and Administration of Adrenocorticotropic Hormone

By Richard Warren, M.D., James Lauridsen, M.D., and John S. Belko, M.S.

The factors influencing alterations in circulating platelets following surgical operations are analyzed. There is probably no bone marrow depression following surgery which might be responsible for the early thrombocytopenia, nor is operative hemorrhage sufficient to do so. Evidence procured from administration of adrenocorticotropic hormone suggests that adrenal hyperactivity, a result of the stress of operation, may play an important part in the early postoperative thrombocytopenia. The later thrombocytosis was, however, not observed following administration of this drug.

Numerous studies on postoperative venous thrombosis indicate that it may be in part due to alterations in the coagulation mechanism induced by the operation rather than entirely to the more commonly incriminated factors of venous stasis or roughened endothelium. Of the coagulation factors affected by a surgical operation the platelets have shown the most consistent changes. A thrombocytopenia during the first three or four postoperative days followed by a thrombocytosis during the second postoperative week is the characteristic response to a major operation of the magnitude of partial gastrectomy or pulmonary resection. What part, if any, this platelet tide plays in causing postoperative thrombosis is not known. It is possible, however, that examination of the factors influencing it would point the way to more rational prophylaxis against postoperative thrombosis.

A fall in circulating platelets could be caused by decreased platelet production or by "peripheral" loss. A study by Kerhulas and co-workers of the activity of megakaryocytes in the bone marrow failed to show valid evidence of depression of activity three days after operations. Further work performed in this laboratory has shown no alteration in platelet adhesiveness during that part of the postoperative period (fig. 1). Since Wright has produced evidence which suggests that increased adhesiveness of platelets is indicative of platelet regeneration, this serves as further testimony against a change in bone marrow activity as the cause of the early thrombocytopenia. In further attempts to explain it we have, therefore, directed our attention to a search for possible causes of peripheral platelet losses. Observations on two of them, hemorrhage and the endocrine response of the body to stress, are reported in this communication.

Methods

Platelet Counts. Venous blood was used and diluted with the sucrose, citrate, cresyl blue solu-
tion* modified by Pohle4 after Reese and Ecker.6 Where syringes were used, it was drawn by clean venipuncture through chemically clean needles into chemically clean dry syringes. In the experiments on hemorrhage the blood was allowed to drip from the needle onto a glass slide whence it was immediately aspirated into the pipet and diluted. In the experiments in which the effect of corticotropin (ACTH) was compared with that of operation the blood was oxalated, using 0.5 cc. of a solution containing 2 Gm. of potassium oxalate and 3 Gm. of ammonium oxalate in 500 cc. of distilled water. This 0.5 cc. was allowed to dry in an oven so that each oxalate bottle which was to receive 4.5 cc. of blood contained 0.0002 Gm. of potassium oxalate and 0.0003 Gm. of ammonium oxalate. The blood was drawn to the 0.5 mark of a red cell pipet which was then immediately filled with platelet diluting fluid to the 101 mark making a dilution of 1 to 200. In the case of the oxalated blood the dilution into the pipet was made within one hour after the blood was drawn. The pipets were then shaken for from one to three minutes, some of the mixture discarded and the counting chambers of a standard hemocytometer filled with the mixture. The platelets were allowed to settle for 15 minutes in a humid atmosphere, one compartment 1 mm. square and 0.1 mm. deep was then counted and the result multiplied by 2000 to procure the number of platelets per cubic millimeter of blood.

Multiple counts performed as unknowns on the same normal individual from day to day and on the same blood sample have consistently shown a standard deviation of not greater than 20,000 platelets, a coefficient of variation of not greater than 10 per cent.

Eosinophil Counts. The method of Hunneman and associates8 was used. Venous blood oxalated in the manner described above was drawn into the standard white cell counting pipet to the 1 mark and diluted up to the 11 mark making a 1:10 dilution with a fluid consisting of 0.1 per cent phloxine and propylene glycol mixed in equal volumes with distilled water, as described by Roche, Hills and Thorn2 and Hunneman, Wexler and Westenhaver.6 After allowing 15 minutes for staining, the pipets were shaken for from one to three minutes and both counting chambers of a hemocytometer were filled. The fluid was allowed to settle for 15 minutes in a humid atmosphere and counts were then performed. By counting the eosinophils in eight of the 1 mm.2 segments and multiplying the result by 12.5 the eosinophils per cubic millimeter of blood were found.

The patients studied were individuals undergoing operations of the magnitude of a subtotal gastrectomy or lobectomy none of which lasted longer than four hours nor less than two and in none of which less than one blood transfusion nor more than four were administered. The normal individuals studied were members of the professional staff, laboratory personnel or volunteer patients who were suffering from no active physical disease, such as those convalescing from fractures incurred at least two months before.

**Observations**

**Effect of Hemorrhage on Platelets**

In 11 patients undergoing surgical operation platelet counts were performed immediately before the induction of anesthesia and within 30 minutes after the termination of the operation. Platelet counts were also performed on the blood used for transfusion, the amount of transfused blood being recorded. The amount of blood lost was determined by the method of weighing sponges.10 By comparing the platelet counts before and after operation and arbitrarily setting the blood volume at 6000 cc., a rough estimate was derived of the total number of platelets which actually disappeared from the circulation. By assuming that the blood shed during the operation contained platelets in concentrations indicated by the preoperative platelet count the numbers of platelets lost due to hemorrhage were estimated and from these were subtracted those

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* The diluting fluid consists of sucrose 20 Gm., sodium citrate 5 Gm., brilliant cresyl blue 0.1 Gm., dissolved in 250 cc. of distilled water.
replaced by transfusion. Table 1 indicates the results of the observations and demonstrates that there is a large disparity between the small numbers of platelets of the shed blood and the large numbers which disappeared from the circulation during operation. Actually, as will be shown below, the platelet counts of the blood shed during operation must have been considerably lower than that of the preoperative blood, probably lower even than that of the postoperative blood. This consideration would make the numbers of platelets lost by hemorrhage during the procedure less than recorded and indicates even more strongly that some avenue of platelet losses other than hemorrhage must be sought for.

Table 2 shows the platelet and eosinophil counts of a normal individual bled rapidly of 500 cc. and within one hour transfused with 500 cc. of blood 19 days old, containing 38,000 platelets per cubic millimeter. There is some irregularity of the platelet levels starting four days after bleeding which does not seem to be characteristic of any trend, least of all that of the postoperative state. Since alterations in the platelet count in the direction of a decrease in platelets might conceivably be due to

<table>
<thead>
<tr>
<th>Case</th>
<th>Estimated Change in Total Platelets from Hemorrhage &amp; Transfusion—Billions</th>
<th>Estimated Change in Total Platelets from Platelet Counts—Billions</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>-11</td>
<td>-372</td>
</tr>
<tr>
<td>2</td>
<td>+96.5</td>
<td>-120</td>
</tr>
<tr>
<td>3</td>
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<tr>
<td>4</td>
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<td>-276</td>
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<tr>
<td>5</td>
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<td>-900</td>
</tr>
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<td>6</td>
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<td>-780</td>
</tr>
<tr>
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<td>-14.7</td>
<td>-528</td>
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<tr>
<td>8</td>
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<tr>
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<td>+25</td>
<td>-24</td>
</tr>
<tr>
<td>11</td>
<td>-90.8</td>
<td>-408</td>
</tr>
</tbody>
</table>

Table 2.—Circulating Platelets after Bleeding of 500 cc. and Replacement with Bank Blood

<table>
<thead>
<tr>
<th>Time after Bleeding</th>
<th>Platelets—Per Cent of Normal</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 hours</td>
<td>91</td>
</tr>
<tr>
<td>4 hours</td>
<td>96</td>
</tr>
<tr>
<td>8 hours</td>
<td>96</td>
</tr>
<tr>
<td>1 day</td>
<td>107</td>
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<tr>
<td>4 days</td>
<td>118</td>
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<tr>
<td>8 days</td>
<td>114</td>
</tr>
<tr>
<td>11 days</td>
<td>73</td>
</tr>
<tr>
<td>14 days</td>
<td>108</td>
</tr>
</tbody>
</table>

Fig. 2.—Mean platelet and eosinophil counts in nine patients receiving surgical operations.

Hemodilution as indicated by the hematocrit, it is of interest to mention that a fall in the hematocrit occurring 50 minutes after bleeding had no effect on the platelet count.

Table 3 shows the changes in the platelet counts of three patients who had varying degrees of gastrointestinal hemorrhage as
indicated and again shows them not to be influenced by hemorrhage of such degree.

**Effect of Operative “Stress” on Platelets**

Most previous studies of platelets have concerned themselves little with the period during and within the first few hours after operation. Before observing the effect of the stress induced by operation by simulating this effect with corticotropin, it is appropriate to describe better than has heretofore been done the platelet changes, not only from day to day following operation, but from hour to hour following the onset of operation during the operative day. Counts were therefore done pre-operatively, each hour for four hours after the start of operation, every two hours for the next four hours and daily thereafter on nine patients undergoing operations of the standard magnitude described. Figure 2 presents the means of the results. It can be seen that there is a sharp drop of nearly 40 per cent in the platelet count occurring maximally between the second and third hours after onset of operation. The curve then, after a slight rebound, remains low and joins the characteristic previously described curve from the first postoperative day.

It can be seen that hematocrit values alter so little during the period of maximal platelet change that it is doubtful whether any hemo-dilution can be invoked as the cause of the observed results.

**The Effect of Corticotropin on Platelets**

The effect on the body of the stress of the operative procedure was then studied by the injection of corticotropin in varying doses by varying routes. Initial studies were performed using corticotropin in 25, 50, 75 and 100 mg. doses by the intramuscular route. In the smaller doses slight decreases of between 5 and 20 per cent were noted in the platelet counts. These decreases became maximal between two and four hours after injection. Larger doses in general showed larger drops of the platelet counts. These observations have already been briefly reported.9

For the purposes of further study an intramuscular dose of 80 mg. was then used as one which seemed to be the smallest which would give a maximal response. Platelet counts were made in the early hours and for several days after the administration of this dose to normals. Figure 3 shows three characteristic responses. The characteristic fall, up to 43 per cent in these cases, is manifest. There is perhaps a slight tendency toward an immediate rebound but no later prolonged rise as is true after operation.

The consideration of the proper dose of corticotropin to simulate a surgical operation has led us to believe that a single intramuscular dose is but a poor representation of the endogenous hormone elaborated during the procedure. Moore and Hume11 have suggested that the stress period continues for two or three days after the operation. Accordingly a normal individual was given intravenously and con-
tinuously over a 72 hour period 90 mg. of corticotropin in decreasing concentrations so that he received 45 mg. during the first 24 hours, 30 mg. during the second 24 hours and 15 mg. during the third. Figure 4 demonstrates the platelet and eosinophil responses in this individual. It can be seen that a response is obtained which is nearly similar to that found during the first three days after a surgical operation. There is, however, no thrombocytosis occurring during the second week.

**Discussion**

The data presented seem to indicate that loss of platelets by the degree of hemorrhage entailed and the types of operations studied is not sufficient to cause the observed changes in the platelet counts. They further show that the platelet falls observed during the first three days after operation can be almost exactly reproduced, even down to their hour by hour changes, by the injection of corticotropin in proper dosages and schedules. Data available so far, however, have not shown a thrombocytosis beginning at the end of the first "postoperative" week after corticotropin, as after operation. Further observations on this score are needed, since if they corroborate these initial ones they will render less tenable the theory that the thrombocytosis of the second week is a compensatory reaction to the early thrombocytopenia. Another cause of the above-mentioned thrombocytosis must then be sought.

It is of course possible that other factors, not so far studied, operate in the early postoperative period to reduce circulating platelets, namely a specific demand on the part of the operative wound for platelets to help with hemostasis in the small vessels of the operative field as described by Zucker\(^\text{12}\) and intimated by the work of Lutz and co-workers\(^\text{13}\) or possibly a splenic hyperfunction. The use of corticotropin and cortisone to cause thrombocytosis in thrombocytopenic purpura, thought by many to be one form of hypersplenism, would at the present time make the splenic theory an undesirable one.

The mystery surrounding the movements of the formed elements of the blood are no-

where greater than in relation to platelets. We have no better idea of where the platelets go after operation or administration of corticotropin than of where the eosinophils disappear to. In the case of the surgical operation, however, it is our impression, derived from evidence of platelet regeneration in the bone marrow and from increased platelet adhesiveness in the late postoperative period, that platelets have been actually destroyed, not hidden away in some lagoon of the circulation. The same may not be true after corticotropin administration.

**Summary**

1. Normal individuals losing by hemorrhage 500 cc. of blood were observed to show no change in circulating platelets whether or not the blood shed was replaced with bank blood. No significant alterations in circulating platelets were observed after major gastrointestinal hemorrhage. These results indicate that hemorrhage incurred during operation has little effect on the altered platelet count observed during the postoperative period.

2. The administration of corticotropin exactly mimicked the postoperative thrombocytopenia found during the first three postoperative days. The later thrombocytosis, which may admittedly be the most important element in causing venous thrombosis, could not be so reproduced.

3. Theoretic causes for the thrombocytosis of the second postoperative week are discussed.

**Sumario Español**

Se analizan los factores que influencian las alteraciones de las plaquetas en la circulación luego de operaciones quirúrgicas. No hay depresión de la médula ósea después de operaciones que pueda explicar la temprana trombopenia ni tampoco la hemorragia operatoria es suficiente para causarla. Evidencia obtenida por medio de la administración de la hormona adrenocorticotrópica sugiere que la hiperactividad adrenal, resultado de esfuerzo de la operación, pueda jugar un papel importante en la temprana trombopenia postoperatoria. La trombocitosis que se observa mas tarde, sin embargo, no fue observada luego de la administración de esta droga.
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