THE BLOOD PLATELETS

THE RATE OF THEIR UTILIZATION IN THE CAT

By John S. Lawrence, M.D., and William N. Valentine, Captain, M.C., A.U.S.

I. INTRODUCTION

Of all the formed elements of the blood the platelet has been the most difficult to study. This has been largely due to its extreme lability in shed blood and the consequent technical difficulties associated with handling it. In particular, there has been little or no valid conception of how long the average platelet may be expected to survive in the intact animal. Estimations of the average rate of platelet utilization have been derived almost entirely from the observed rate of regeneration in animals rendered acutely thrombopenic by one or another means—a method whose validity is open to serious question. In addition to these observations, Duke in 3 patients with thrombopenic purpura was able to make an indirect estimate of platelet life span by noting the duration of improvement in hemostasis following direct transfusion. It is also possible to arrive at some rough estimate of the rate of platelet utilization if one accepts the figures of Howell and Donahue on platelet counts made simultaneously from venous and arterial blood. However, these findings were not confirmed by Fidlar and Waters, or by Tocantins and Bradshaw. This brief pertinent literature will be reviewed presently, but one is forced to conclude that there is little in the way of concrete data to support more than a conjecture.

It is the object of this report to present data obtained by in vivo studies on the cat relative to the average rate of platelet utilization in that animal. In principle, the method has been as follows. A cat is rendered chronically thrombopenic by means of radiation and then cross circulated via carotid to carotid anastomoses with a normal animal. After equilibrium has been established the thrombopenic animal possesses in the neighborhood of half the platelets originally belonging to the normal cat. Each animal is then returned to its own circulation and the rate of disappearance of the cross circulated platelets is followed by repeated counts. The methods employed will be discussed in detail in a subsequent section. In effect, therefore, the results are analogous to those obtained by following the rate of disappearance of transfused erythrocytes, the radiated animal in this instance having been rendered incapable of producing more than negligible numbers of platelets. Cross circulation serves merely as a device to give the thrombopenic animal a
massive direct transfusion rich in platelets, the platelets never leaving the vascular system or contacting other than an endothelial surface.

II. REVIEW OF THE LITERATURE

Only isolated estimates of the in vivo rate of utilization of the blood platelet in either animal or man appear in the medical literature. The majority of these reports are referred to by Tocantins in his excellent monograph on the mammalian platelet.

The earliest report of which the authors are aware is that of Duke in 1910. This investigator found that the hemorrhagic diathesis in 3 patients with idiopathic thrombopenia was largely relieved by direct blood transfusion. In 1 case the platelet count was reportedly raised from 3,000 to 123,000 platelets per cubic millimeter and in another from 20,000 to 89,000 per cubic millimeter. The transfused platelets had almost completely disappeared within three days. Duke concluded that while these platelets may conceivably have been prematurely destroyed by the disease process, they probably were shortlived bodies whose utilization might be as rapid as one-fourth the total number per day. He also mentioned that experimental results of platelet transfusion in animals rendered thrombopenic by benzol suggested a rapid rate of platelet disappearance. Published details on these experiments could not be found. Duke, again the following year, reported results obtained by observing the rate of platelet regeneration in dogs made thrombopenic by the removal of blood and its reinjection after defibrination. On the average, the rate of regeneration under these circumstances amounted to around one-fifth the entire number in the blood per day. Firket (quoted by Tocantins) obtained essentially similar results in animals rendered thrombopenic by both the defibrination technic and after saponin injections. Similar rates of regeneration have also been noted by Bedson and Tocantins after thrombopenia induced by the injection of antiplatelet serum. In this connection, however, it is also interesting to note that an increase of 400,000 to 1,000,000 platelets per cubic millimeter in twenty-four hours has been observed after splenectomy in patients with thrombopenia.

In 1916 Minot and Lee noted marked improvement in the coagulation time of hemophilic patients after transfusion. This lasted for approximately three days. The authors felt that this was probably due to the introduction of normal platelets and that the clinical results were in corroboraton of the findings of Duke. Of course, more recent work has indicated that improvement in hemostasis in hemophiliacs after transfusion is not due to introduced platelets per se.

Shause, Warren, and Whipple noted the sudden disappearance of platelets in dogs seven to eight days after they received radiation over the entire bony skeleton. Since megakaryocytes were virtually all destroyed by the amount of radiation given, it was suggested that the life of the platelet in the peripheral blood might be around seven to eight days.

Howell and Donahue in 1937 found arterial blood consistently to contain a larger number of platelets than simultaneously removed samples of venous blood. For this reason, and because of evidence interpreted as indicating that platelet production in abundance took place in the lungs, it was concluded that new platelets...
are added to the blood in the capillary areas of the lungs and that a corresponding
destruction of platelets occurs as the blood passes through the capillary areas of the
systemic circulation. Using the figures given, it is apparent that this would involve
a complete replacement of the entire platelet mass in approximately ten complete
circulations of the blood. However, Tocantins and Bradshaw (reported by Tocantins) found inconstant relationships between the platelet counts of arterial and
venous blood. Fidlar and Waters were likewise unable to confirm either a signifi-
cant differential in the platelet levels of arterial and venous blood or to obtain
satisfactory evidence of important platelet production in the lungs.

In view of the conjectural nature of the rate of platelet utilization in either man
or animal as suggested by the scanty evidence available in the medical literature,
it appeared desirable to investigate the problem by some direct method in which
the actual rate of disappearance of normal platelets could be measured.

III. METHODS

Normal cats were rendered thrombopenic by repeated exposures to known
amounts of radiation and then under nembutal anesthesia cross circulated via
carotid anastomoses with nonradiated animals. The full details of the method of
establishing the cross circulation are given elsewhere. Suffice it to say here that
platelets traversing the anastomoses have a continuous endothelial lined pathway
in their passage from one animal to the other.

Cross circulation was allowed to function for a sufficiently long time to establish
equilibrium and then each animal returned to its own circulation. In eight satisfac-
tory experiments of this nature it was possible to elevate the platelet count of the
previously thrombopenic cat anywhere from 100,000 to 450,000 per cubic milli-

m...
cance. Results were further checked by examination of Wright stained cover slip preparations for the number of platelets present. Glassware was scrupulously cleaned and any preparations rendered unsatisfactory by contact hemolysis of erythrocytes discarded.

In connection with the cross circulation experiments, observations were made to determine the physiologic variations of platelet counts made by this method on normal cats. In 121 counts made in this manner on normal animals the average platelet count was found to be 422,000 per cubic millimeter. The following percentage distribution was obtained.

<table>
<thead>
<tr>
<th>Platelet Count</th>
<th>%</th>
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<tbody>
<tr>
<td>100,000-200,000 per c.mm.</td>
<td>0</td>
</tr>
<tr>
<td>200,000-300,000 ”</td>
<td>17.4</td>
</tr>
<tr>
<td>300,000-400,000 ”</td>
<td>32.2</td>
</tr>
<tr>
<td>400,000-500,000 ”</td>
<td>21.5</td>
</tr>
<tr>
<td>500,000-600,000 ”</td>
<td>14.0</td>
</tr>
<tr>
<td>over 600,000 ”</td>
<td>4.9</td>
</tr>
<tr>
<td>100.0</td>
<td></td>
</tr>
</tbody>
</table>

Total dosage of radiation varied somewhat from animal to animal depending on their toleration of the initial exposure and on the satisfactory development of severe thrombopenia. The following factors were kept constant.

- Voltage: 150 K.V.P.
- Milliamperage: 15
- Target distance: 22 inches (to center of cat)
- Filter: Aluminum parabolic plus ½ mm. copper
- Half value layer: 1.1 mm. copper
- Output: 20-35 r per minute with minor variations

In seven of the eight experiments an initial dose of 250 to 350 r whole body radiation (usually 250 r) was given. One week later the animal received a second dose of 200 r to 300 r (usually 200-250 r). Four to five days after the second exposure a third dose of 100 r to 250 r was given (usually 150 r). Total dosage therefore amounted to 650-800 r over an eleven to twelve day period. Animals in good condition at this time but satisfactorily thrombopenic were used for cross circulation. While some animals did not survive the period of preparation, it was surprising how many animals could be obtained in good condition at the time of operation. In one animal, cat number 64, the radiation preparation was considerably more gradual. This animal was used in the first successful experiment when radiation technic for producing chronic thrombopenia was still in the formative stage.

Since these animals were also markedly granulocytopenic it was customary to administer penicillin in saline subcutaneously at intervals throughout the experiment.

In all but one instance the thrombopenic animal was cross circulated with a normal cat. Cat number 234, however, was deliberately cross circulated with an animal splenectomized some ten days previously. The splenectomized animal in this
IV. PRESENTATION OF DATA

The data are presented in tabular and graphic form.

Table 1 shows the base line platelet level of each animal prior to cross circulation, the level attained immediately after return to independent circulation, and the platelet levels at approximately twenty-four hour intervals for five days thereafter. It is of course impossible to tabulate all the platelet counts performed on each animal. It was customary to perform at least four sets of duplicate platelet counts in the few hours after cross circulation. These showed some fluctuation for a few hours, probably incident to vascular readjustments associated with return to independent circulation and recovery from anesthesia. The first count after cross circulation alone is given for purposes of conserving space. It is reasonably representative. It was also customary to do at least three sets of duplicate counts every day on each animal until the pre-cross circulation base line had again been reached. Again, data are given only on that count closest to twenty-four hours or some multiple thereof after the time cross circulation was discontinued. The counts tabulated are in complete agreement with the rest of the data.

Figure 1 shows graphically the rate of disappearance of cross circulated platelets in four of the eight experiments. Each point represents the average of a set of duplicate platelet counts and the fluctuations mentioned previously can be readily noted. These four experiments were selected for purposes of illustration since the peak platelet level attained varied by approximately equal intervals from around 100,000 per cubic millimeter to better than 400,000 per cubic millimeter.

Table 2 indicates the average rate of platelet utilization per cubic millimeter per hour computed for each animal. It is obvious, in view of some fluctuations in the
counts soon after cross circulation and in view of an insufficient number of counts
to determine the exact time after cross circulation that the animal returned to its
preoperative base line, that the figures given represent an approximation. The plate-
let level attained after cross circulation was arbitrarily designated as the first count

Figure 1: Rate of Disappearance of Cross Circulated Platelets in Four of the Eight Experiments
Each point represents the average of duplicate platelet counts made at the indicated time.

Table 2 — Individual and Average Group Rate of Platelet Utilization

<table>
<thead>
<tr>
<th>Cat No.</th>
<th>Average Number of Platelets Utilized per c. mm. per hour</th>
</tr>
</thead>
<tbody>
<tr>
<td>154</td>
<td>4,613</td>
</tr>
<tr>
<td>186</td>
<td>2,635</td>
</tr>
<tr>
<td>64</td>
<td>2,062</td>
</tr>
<tr>
<td>152</td>
<td>1,659</td>
</tr>
<tr>
<td>132</td>
<td>1,804</td>
</tr>
<tr>
<td>236</td>
<td>2,861</td>
</tr>
<tr>
<td>221</td>
<td>2,811</td>
</tr>
<tr>
<td>194</td>
<td>1,894</td>
</tr>
<tr>
<td>Total</td>
<td>10,339</td>
</tr>
<tr>
<td>Average for group =</td>
<td>2,542 platelets utilized per c.mm. per hour.</td>
</tr>
</tbody>
</table>
after return to independent circulation; the time of return to the base line was arbitrarily designated as the time of the first platelet count under 50,000 per cubic millimeter.

V. ANALYSIS OF RESULTS

From an examination of table 1 it is apparent that no hard and fast rule can be laid down regarding the rate of platelet utilization which will apply to each animal. This is of course to be expected in the light of the fact that platelets when utilized are almost always destroyed. It would indeed have been surprising had it been possible to note a perfectly uniform rate of fall in one individual, much less uniform rates of disappearance in different individuals. Demands for platelets must of necessity vary rather widely in different animals or in the same animal at different times.

Despite lack of complete uniformity from experiment to experiment, certain important generalities or trends show good correlation. In every instance platelets disappeared from the circulation in a steadily progressive manner with significantly lower counts noted in each successive twenty-four hour period. In many instances, as in three of the four experiments graphed on figure 1, these rates of disappearance roughly paralleled each other, depending on the height of the initial level after cross circulation. Further, there was a general trend for animals receiving relatively slight elevations in platelet count to return to their preoperative base line faster than in those receiving marked elevation in platelet count. The chief exception to this is cat number 254 in which the highest platelet level of the group was obtained. Here, the rate of fall of the cross circulated platelets was relatively precipitous and the average rate of platelet utilization was about twice as great as the average for the rest of the group. This animal alone received platelets from a splenectomized donor. Whether the platelets from this donor had been altered in some manner as a result of splenectomy can only be a matter of speculation.

However, the important feature of the presented data is that it took two to slightly more than four days for cross circulated platelets to disappear from the recipient. Since preliminary observations on normal cats indicated that around 17 per cent had platelet counts between 200,000 and 300,000 per cubic millimeter and 32 per cent had platelet counts between 300,000 and 400,000 per cubic millimeter, the counts obtained in experimental animals were within the physiologic range of about 50 per cent of the normals tested by identical methods. This indicates that under the conditions of this experiment the entire platelet mass in the cat requires replacement every three to five days.

VI. DISCUSSION

The data presented constitute the first observations on animals in which rate of platelet utilization has been directly measured. It is somewhat surprising that the results obtained are in rather close accord with estimates made indirectly from observations on the regeneration rate of platelets after experimental thrombopenia. One would expect the rate of regeneration under the intense stimulus of severe platelet depletion to be a poor index indeed to the normal rate of production
(and hence the normal rate of destruction) in the normal animal. The findings are also in rather close agreement with those of Duke. The data obtained are not, however, consistent with the observations of Howell and Donahue.

A turnover in the entire number of platelets in the body within a period of three to four days is probably a minimal estimate. That is, turnover in the completely normal animal may be slightly slower. The animals employed had received radiation and did have an operative incision to heal. In no case was any infection noted despite close check and in no animal was there apparent loss of blood from any source until marked thrombopenia had recurred. However, it cannot be denied that there may have been some increase in demand for platelets in animals under these conditions. There is no evidence that such an increase in demand would be great if it existed at all.

The sequence of events at the time of operation was dramatic testimonial to the possible role of the platelet in hemostasis. When the neck incision was made in the thrombopenic cat persistent oozing from traumatized vessels was a major problem. Hemostasis was difficult to secure and often oozing persisted to some extent despite all efforts. However, very shortly after cross circulation was established all oozing ceased spontaneously. The field at closure was invariably dry and no difficulty was experienced with bleeding from the wound after operation. It was only three to four days later when the platelet count was again very low that purpuric manifestations recurred. These became progressively worse and were often the cause of death within the following few days. Of course, it is recognized that the platelet may have been only one factor in producing this effect on hemostasis. Indeed, it could conceivably have not had any effect at all. The basis for this statement is the observation which has been made repeatedly by us at the time of splenectomy in idiopathic thrombopenic purpura. At the time of splenectomy abnormal oozing of blood frequently stops abruptly when the splenic pedicle is clamped in spite of the fact that the number of platelets in the blood remains unchanged. Thus, the effect on hemostasis in our animal could have been due to some other substance or substances in the blood than the platelet. However, the correlation between the bleeding tendency and the platelet level was so close that we feel the platelet must have been at least one important factor with regard to the bleeding.

Care has been taken to refer only to "rate of platelet utilization" rather than to platelet "life span." Since the platelet, unlike the erythrocyte, is destroyed when used, data on rate of disappearance of transfused platelets are a clue mainly to platelet demands. They disclose nothing as to how long a platelet might survive without spontaneously disintegrating if there were no demands for its use.

In the light of the experiments recorded here, the observations of Duke in human beings deserve some further comment. If they could be substantiated, massive direct transfusion would certainly be of importance in tiding patients with idiopathic thrombopenic purpura over critical periods of bleeding or in preparing them for splenectomy. Significant improvement in hemostasis might warrant the use of the more inconvenient direct transfusion rather than the customary method of transfusing stored blood in which platelets are rapidly destroyed. In the experiments reported by Duke, neither the exact amount of blood transfused nor the
details of the direct method employed are indicated. In one of the experiments the platelet level was raised approximately 120,000 per cubic millimeter (3,000–123,000). If the donor blood contained a normal number of platelets, one would have to assume that the recipient was given approximately 2500 cc. of blood. Whether such large quantities of blood were given cannot be determined from his data. However, we have recently given a direct transfusion of 2000 cc. within twenty-four hours to a severely thrombocytic patient with aplastic anemia (platelet count below 10,000 per cubic millimeter). The multiple syringe method was used and 1500 cc. of the transfused blood given within a three hour period. The other 500 cc. had been given about fifteen hours previously. The platelet count was never detectably raised and no improvement in purpuric manifestations occurred. Bleeding from the nose, present before transfusion, continued unabated afterwards. The failure to obtain any elevation of platelet count or improvement in hemostasis is unexplained. Experiments are contemplated to determine if possible what happens to the blood platelet during its brief sojourn outside the body during a direct transfusion. It should be emphasized, however, that direct transfusion methods currently in use are not comparable to the continuous endothelial anastomoses of the animal experiments. One experiment, of course, proves nothing, and more trials of a similar nature employing other types of direct transfusion apparatus will have to be made. Experiments of this type are being carried out as suitable patients appear in the clinic. Careful observations on a few patients should give a definite answer as to whether the platelet level of the human being can be raised significantly with a corresponding beneficial effect on hemostasis in patients with thrombocenia by means of direct transfusion of blood. If such should prove to be the case, direct rather than indirect transfusion should be used in thrombocenic individuals.

VII. SUMMARY

1. The rate of utilization of blood platelets in radiated, thrombocytic cats has been measured directly. Thrombocytic animals, incapable of significant platelet regeneration, were cross circulated via carotid to carotid anastomoses with normal animals. After return to independent circulation the rate of disappearance of cross circulated platelets was measured by periodic counts.

2. By this method it was possible to elevate the platelet count anywhere from 100,000 to around 400,000 per cubic millimeter. The highest count obtained followed cross circulation with a splenectomized animal. In most instances the platelet level attained was within the physiologic range of that found for normal cats by the same method.

3. The cross circulated platelets gradually disappeared from the circulation over a two to a slightly more than four day period. Under the conditions of this experiment the entire platelet mass would have to be replaced therefore every two to five days. The same figures probably apply within narrow limits to the normal cat.

4. The average rate of platelet utilization was approximately 2500 per cubic millimeter per hour. In seven of eight experiments the rate of disappearance varied
from about 1600 per cubic millimeter per hour to about 2800 per cubic millimeter per hour. In the experiment using a splenectomized donor the rate of disappearance was about double the average for the rest of the group.

5. Attention is called to possible therapeutic implications of these findings in idiopathic thrombopenic purpura.

REFERENCES

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