Isoimmunity to Blood Platelets in the Rabbit

By Yves Borel, Mario Baldini and Shirley Ebbe

It has long been known that blood platelets are isoantigenic. Serum isoantibodies reacting with platelets have been repeatedly demonstrated in patients who have received multiple blood transfusions, and in multiparous women. The incidence of isoimmunity to blood platelets in multitransfused patients has been studied mainly by the agglutination, the anti-human globulin consumption, the indirect Coombs and the complement fixation tests. By these technics, the percentage of multitransfused patients sensitized to homologous platelets has been found to be relatively low, and the conclusion has been derived that isoimmunity to blood platelets in humans is a rather uncommon occurrence.

Since the degree of sensitivity of the various serologic technics used for the detection of platelet antibodies is unknown, the assumption that a negative result would exclude the presence of antibody in the patient's circulation may not be correct. The present investigation was done to test this hypothesis. Rabbits were repeatedly transfused with homologous blood and the development of platelet isosensitization was studied by determining the survival of radioactively labeled donor platelets infused in the recipient animals. Simultaneously, the presence of platelet isoantibodies in the serum of the multitransfused animals was investigated by the agglutination method described by Dausset et al.

The results obtained demonstrated that after 3 to 12 blood transfusions, reduction in survival of homologous platelets in the recipient animals almost invariably occurred, while platelet isoantibodies could be serologically demonstrated in but a few of those animals which had received the highest number of blood transfusions. The persistence of platelet isoantibodies in the multitransfused rabbits was also investigated.

Materials and Methods

Outbred white or black rabbits of 2.5 to 4.5 Kg. body weight were used in these studies. As a rule, the rabbits were arranged in pairs, one being the donor, the other the recipient. Donor and recipient were selected of different color. Every 2 to 3 days, 9 ml. of heart blood were drawn from the donor animals into a siliconized syringe containing 1 ml. of sodium EDTA solution* and transfused immediately into the ear vein of the respective recipients. After 3, 5, 6, or 12 blood transfusions, survival of the donor's platelets labeled in vivo with P was measured in the recipient animal. Simultaneously, the serum of the recipient was tested for the presence of platelet agglutinins using platelets from the respec-

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Submitted Nov. 30, 1962; accepted for publication Feb. 9, 1963.

*One and a half per cent disodium ethylenediaminetetraacetate dihydrate (EDTA) in 0.7 per cent sodium chloride.
### Table 1.—Comparison between Platelet Survival and Agglutinin Test in the Detection of Isoimmunity to Blood Platelets in Multitransfused Rabbits

<table>
<thead>
<tr>
<th>Rabbit No.</th>
<th>Number of blood transfusions</th>
<th>Platelet donor</th>
<th>Maximum per cent recovery</th>
<th>Survival time* (hours)</th>
<th>Agglutinin Test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Normal serum</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Hetero-immune</td>
</tr>
<tr>
<td>1</td>
<td>3</td>
<td>specific</td>
<td>57</td>
<td>45</td>
<td></td>
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<tr>
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<td>3</td>
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<td>89</td>
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<td>56</td>
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<td>83</td>
<td>51</td>
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<td>5</td>
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<td>62</td>
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<td>6</td>
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<td>81</td>
<td>72</td>
<td></td>
</tr>
<tr>
<td>7</td>
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<td>55</td>
<td>55</td>
<td></td>
</tr>
<tr>
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<td></td>
<td>non-specific</td>
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<td>52</td>
<td></td>
</tr>
<tr>
<td>13</td>
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<td>5</td>
<td>4</td>
<td>+</td>
</tr>
<tr>
<td>14</td>
<td></td>
<td>non-specific</td>
<td>12</td>
<td>5</td>
<td>+++</td>
</tr>
<tr>
<td>15</td>
<td>12</td>
<td>non-specific</td>
<td>60</td>
<td>18</td>
<td></td>
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<tr>
<td>16</td>
<td></td>
<td>non-specific</td>
<td>55</td>
<td>24</td>
<td>++</td>
</tr>
<tr>
<td>17</td>
<td></td>
<td>non-specific</td>
<td>50</td>
<td>60</td>
<td></td>
</tr>
</tbody>
</table>

Normal values (in 13 experiments): Survival of homologous platelets 86.5, 72 (70.8 to 113, 58 to 89).

*The time at which 10 per cent of the peak value was reached.

"Specific" = specific platelets, "Non-specific" = non-specific platelets used in the survival study.

tive donor. On several occasions, as specified in the tables and graphs, platelets from a rabbit other than the sensitizing donor were used for the platelet survival study; these platelets were defined as non-specific platelets, while specific platelets, i.e., platelets from the sensitizing donor, were used in most experiments. Three rabbits were sensitized with blood from more than one donor. In each study of platelet agglutinin, platelets from the same donor animal were used as for the platelet survival study.

The individual experiments were as follows (table 1):

1. Six recipient rabbits received three blood transfusions. Platelet survival studies were done with specific platelets in four experiments while non-specific platelets were used in two experiments (No. 5, 6). These studies were performed 9 to 11 days after the first transfusion.

2. One rabbit received five blood transfusions. Specific platelets were used in the platelet survival study which was done 17 days after the first transfusion.

3. Five rabbits received six blood transfusions. For the survival studies of homologous platelets done 15 to 32 days after the first transfusion, specific platelets were used in two experiments (No. 8, 9) and non-specific platelets in two other experiments (No. 10, 11). The fifth rabbit (No. 12) was sensitized with blood from three different donors. Platelets used for the survival study were from a fourth donor animal.

4. Five rabbits received 12 blood transfusions. Platelet survival studies were done 36 to 46 days after the first transfusion using non-specific platelets in all the animals of this group. Two of the five rabbits (No. 16 and 17) were sensitized with blood from several donors (four donors for one rabbit (No. 16) and six donors for the other (No. 17). The
non-specific homologous platelets for these two experiments were collected from the last donor animal used.

**Platelet Survival**

Viability of the homologous platelets in the multitransfused rabbits was measured using platelets labeled in vivo with P³². The donor animal was injected with sodium phosphate-P³², 200 μc./Kg./body weight. Three days later, 60 to 90 ml. of its blood were collected by cardiac puncture into EDTA anticoagulant (20 per cent by volume), the platelets were separated by differential centrifugation in the cold and the platelet button, suspended in 4 ml. of platelet-poor plasma and 4 ml. of saline (containing 0.02 per cent Triton*), was injected intravenously into the recipient. Platelet radioactivity in the circulation of the recipient animal was followed by taking 5-ml. blood samples at 30 minutes, 1 hour and 4 hours after injection; twice the following day and once daily thereafter.

The results were expressed as per cent recovery—that is, in percentage of the total platelet radioactivity infused. In this manner a destruction of platelets occurring soon after infusion and before the first sample was taken could be detected. The survival time of the infused platelets was determined from the time at which the curve obtained by interpolation of the experimental values reached 10 per cent of its maximum value. Details of this technic for the study of survival of homologous platelets in rabbits were previously described by Ebbe et al.¹⁵

**Platelet Agglutinins**

The method of Dausset, Colin and Colombani¹¹ was followed for the study of platelet warm agglutinins. Serum from the multitransfused animals was first inactivated by heating at 56 C. for 30 minutes, then absorbed for 15 minutes with barium sulphate (0.1 Gm. of BaSO₄ for 1 ml. of serum). The platelets used in the test were from the same donor animal whose platelets had been used for the survival study. The platelets were separated from a 9-ml. sample of blood obtained by cardiac puncture with a syringe containing 1 ml. of a 5 per cent solution of EDTA in saline. The platelets were then washed three times in phosphate buffer and resuspended in saline to a final concentration of 1 million platelets per cu. mm. All the details of the original method were followed, with the only difference that the preparations were kept on the Kline agitator for 2 hours instead of 1. Each test was carried out in duplicate and with two duplicate controls: in one control a potent heteroimmune (guinea pig) anti-rabbit-platelet serum was used, while in the other control, serum from a normal (non-transfused) rabbit was added. The latter control test with normal rabbit serum was constantly negative, whereas when the heteroimmune serum was used, agglutination was always obvious. The degree of agglutination obtained in these studies was expressed with (+) to (+++) or with (−) when no agglutination could be seen.

**RESULTS**

**Survival of Homologous Platelets in Normal Rabbits**

In all the charts included in this paper, a shaded area can be noted which represents the average survival curve of normal homologous platelets labeled in vivo with P³² and infused in normal, non-sensitized recipient rabbits. This area encloses one standard deviation on either side of the mean of the values obtained in 13 experiments, the details of which have been published elsewhere.¹⁵ As seen in the illustrations here included, after a short initial sluggish phase the normal radioactive platelets disappeared gradually from the circulation. The average survival time, i.e., the time at which 10 per cent of the

Fig. 1.—Effect of six infusions of platelet-rich plasma on survival of homologous platelets in rabbits.

value at the peak of the curve was reached, was 72 hours, varying from 58 to 89 hours. The average value of maximum per cent recovery—the peak value of platelet radioactivity in the recipient’s circulation expressed in per cent of the total platelet radioactivity infused—was 86.5, varying from 70.8 to 113. By this technic, platelet survival values are relatively accurate and reproducible. In fact, in the 13 experiments performed in normal rabbits, practically all the platelet survival curves fell within the relatively narrow area enclosed by one standard deviation. This area was used in the present investigation as the normal control value against which the survival curves obtained with homologous platelets infused in multitransfused recipients were compared.

Preliminary Experiments on Platelet Isosensitization in Rabbits

In four experiments, rabbits were infused six times in the course of 3 weeks with fresh platelet-rich plasma collected from 9 ml. of blood from their respective donor animals. Seven days after the last infusion, survival of homo-
Fig. 2—Effect of multiple blood transfusions on survival of homologous platelets in rabbits.
Isoimmunity to Blood Platelets was studied. As seen in figure 1, repeated intravenous infusions of platelet-rich plasma produced considerable reduction in survival of the subsequently infused homologous platelets in two experiments as demonstrated by the lower than normal values of maximum recovery and the shorter survival time. The two other experiments were also significant, although in one, only the per cent recovery was below the normal range, while in the other only the survival time was shorter than normal. The increased rate of destruction of homologous platelets in the presence of a normal platelet count in these rabbits which had been transfused several times with homologous platelet-rich plasma was interpreted as due to the development of platelet isoantibody. Thus, the rabbit was considered to be a suitable experimental animal for studies on the occurrence of platelet isoimmunization after multiple transfusions of fresh blood.

Survival of Homologous Platelets in the Multitransfused Rabbits

The results obtained in these experiments are summarized in figure 2 and table 1. It can be seen that in the rabbits sensitized by 3 to 12 transfusions of whole blood, the survival of homologous platelets was reduced below normal values in almost all experiments and a definite relationship existed between number of transfusions and degree of reduction in survival of the homologous platelets. It can furthermore be noted that shortening in survival time was usually accompanied by reduction in maximum recovery and, often, by a more curvilinear shape of the curve. Occasionally, however, the two parameters, maximum recovery and survival time, were not equally significant in expressing reduction in viability of the infused homologous platelets. This was particularly observed in those experiments in which the rabbits were sensitized with smaller numbers of transfusions and there was only a moderate degree of reduction in viability of the homologous platelets.

In the six rabbits which received three blood transfusions, the four survival curves obtained with specific homologous platelets were definitely abnormal with the exception of one curve (No. 4) in which the value of maximum per cent recovery was normal and the survival time appeared only moderately shortened (51 hours). In the two experiments in which non-specific platelets were used, the platelet survival curve was normal on one instance (No. 6) and abnormal in the other (No. 5). The actual values of platelet survival time and maximum per cent recovery are reported in table 1.

In the one rabbit sensitized with five blood transfusions, the survival time of homologous specific platelets was only 46 hours (minimum normal value, 58 hours), while the value of maximum recovery was normal.

Viability of homologous platelets was definitely reduced in all the five rabbits which had received six blood transfusions. Survival time and maximum recovery values were more reduced in the two experiments in which specific platelets were used. In none of the rabbits sensitized with 12 blood transfusions could the survival of specific homologous platelets be determined because of death of the donor animals due to repeated heart punctures. Although the
Fig. 3.—Persistence of platelet sensitization.
results were obtained with non-specific platelets they are, however, clear. In two of the three rabbits each sensitized with blood from one single donor, viability of the non-specific homologous platelets reached minute values with survival times of 4 and 5 hours, and maximum recovery of 5 and 12 per cent respectively. In the third animal (No. 15), the platelet survival time was also drastically reduced (18 hours) while the value of maximum recovery was 60 per cent (minimum normal value, 70.8 per cent). Two other recipient animals in this group (No. 16 and 17) had been transfused 12 times with blood from several donors and the platelet survival study was done with platelets from the last donor. Viability of the homologous platelets was also definitely reduced in these two experiments although to a lesser degree than in the three animals transfused with blood from one single donor. Values of survival time were 24 and 60 hours and values of maximum recovery were 55 and 50 per cent respectively.

Platelet Agglutinins in the Serum of the Multitransfused Rabbits

In none of the rabbits transfused three, five and six times with homologous blood could platelet isosensitization be detected by the platelet agglutinin study. The test gave positive results only with the serum of three of the five rabbits transfused 12 times. Of the two rabbits in the "12 transfusions" group (No. 13 and 14), in which viability of the non-specific homologous platelets was minimal, one demonstrated a strongly positive (+++ ) agglutinin test, while the serum from the other showed only a mildly positive (+) reaction. For two other rabbits in this group (No. 16 and 17) transfused with blood from several donors, the agglutinin test was moderately positive (++ ) with the serum of one and negative with the serum of the other (table 1).

The Persistence of Platelet Isosensitization

In three of the multitransfused rabbits, the platelet survival and the platelet agglutinin studies could be performed again after relatively long periods of time during which they were not exposed to further blood transfusions. These results are reported in figure 3 and table 2.

The first rabbit (No. 7) which had received five blood transfusions was restudied after 2½ months. In this second study, non-specific platelets were used, while specific platelets had been used in the first study. Yet, survival values of the homologous platelets were found to be further reduced after this period of time. This result indicated that platelet isosensitization had persisted in the rabbit during the 2½-month interval. The further reduction in survival of the homologous platelets was probably due to the fact that when the first platelet survival was determined, the rabbit had only received five blood transfusions, while at the time of the second study the rabbit had received in addition one infusion of pure platelets. Platelet agglutinins were never found in the serum of this animal.

The two other rabbits in which a second study could be performed belonged to the "12 transfusion" group, and 15 months had elapsed from the time of
Table 2.—The Persistence of Platelet Isoimmunity in Multitransfused Rabbits

<table>
<thead>
<tr>
<th>Rabbit No.</th>
<th>Number of blood transfusions</th>
<th>Time period after sensitization</th>
<th>Survival of Homologous Platelets</th>
<th>Agglutinin Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>5</td>
<td>7 days</td>
<td>Maximum per cent recovery</td>
<td>95</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.5 months</td>
<td>Survival time* (hours)</td>
<td>46</td>
</tr>
<tr>
<td>14</td>
<td>12</td>
<td>12 days</td>
<td></td>
<td>12</td>
</tr>
<tr>
<td></td>
<td></td>
<td>15 months</td>
<td>+ + +</td>
<td>6</td>
</tr>
<tr>
<td>15</td>
<td>12</td>
<td>20 days</td>
<td>58</td>
<td>48</td>
</tr>
<tr>
<td></td>
<td></td>
<td>15 months</td>
<td></td>
<td>58</td>
</tr>
<tr>
<td>Normal values (in 13 experiments)</td>
<td></td>
<td></td>
<td>86.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>72</td>
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</tr>
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</table>

*The time at which 10 per cent of the peak value was reached.

The first investigation. For the platelet survival study, blood was collected from two non-specific donors and platelets were pooled before infusion. The results demonstrated clearly an improvement in platelet survival values after the 15-month interval. In the rabbit in which survival of homologous platelets had demonstrated minute values at the time of the first study, the platelet survival time was 48 hours and the maximum recovery was 58 per cent after 15 months. While the platelet agglutinin test had been strongly positive (+++) at the time of the first platelet survival, it was found to be negative when the second platelet survival was determined. The other rabbit, in which the survival of homologous platelets had only been moderately reduced soon after the 12 blood transfusions, demonstrated a perfectly normal platelet survival after the 15-month interval. The platelet agglutinin test which had given a negative result at the time of the first study remained negative.

**DISCUSSION**

The reduction below normal values in viability of homologous blood platelets infused in the multitransfused rabbits was interpreted as due to an anti-platelet isoantibody which had arisen in the recipient animals as a consequence of the repeated blood transfusions. Although survival of the recipient's own platelets could not be measured, there was indirect evidence that this was not affected:

1. The platelet counts in the recipient animals remained always within the normal range throughout the experiments.

2. If the anti-platelet factor present in the recipient animals had also attacked their own platelets, the platelet specific activity (counts per minute per 10⁹ platelets) after infusion of the labeled donor's platelets would have not rapidly changed. On the contrary, a rapid change in platelet specific activity could be observed in many of the survival curves (fig. 2) and, in the presence of a normal count, it indicates that the homologous labeled platelets were selectively destroyed.
ISOIMMUNITY TO BLOOD PLATELETS

That reduction in survival of the homologous platelets was indicative of a platelet isoantibody in the multitransfused animals is supported also by the following evidence:

1. Survival of the homologous platelets was more markedly reduced in the animals which had received more blood transfusions (table 1).

2. In the serum of some of the animals which had received 12 blood transfusions, a platelet agglutinin could also be demonstrated.

3. The anti-platelet factor in the multitransfused rabbits had broad reactivity, being effective not only against the platelets of their respective blood donors (specific platelets), but also against the platelets of other donors (non-specific platelets), although non-specific platelets usually survived better than specific platelets.

An important finding was that isoimmunity to blood platelets could not be detected by the agglutinin test adopted in these studies until the animals had received a relatively large number of transfusions. Furthermore, the degree of positivity of this test could not be correlated with the degree of reduction in survival of the homologous platelets. In fact, there was practically no difference in values of platelet survival in rabbits No. 13 and 14 of the “12 transfusions” group, being severely reduced in both animals, while the agglutinin test performed respectively with the same platelets used in the survival studies was (+) in one and (+ + +) in the other. A discrepancy could also be seen in the two animals in this group (No. 16 and 17) which were sensitized with blood transfusions from various donors (table 1). In the repeat studies 15 months after sensitization (table 2), rabbit No. 14 still had lower than normal survival values for homologous platelets but the agglutinin test had become negative.

The platelet agglutinin technic in the present experiments was that of Dausset et al. for platelet warm agglutinins. In the experience of these authors this technic is more sensitive than others for the detection of platelet isoimmunity in humans.11 In this respect, however, it should be mentioned that numerous technics for platelet antibodies have been proposed in the last decade and there is no unanimous agreement on which technic is more sensitive and accurate for the study of platelet isoimmunity in sensitized patients.12-14 It is possible that different serologic technics detect different types of antibody and it cannot be excluded that by using other serologic technics or a battery of methods, isosensitization in the multitransfused rabbits could have been detected more frequently. However, when various serologic methods, either single or multiple, were used in the study of platelet isoantibodies in multitransfused patients, results did not greatly differ (see further). Therefore, the impression gained from our experiments is that the determination of survival of homologous platelets is, at present, the most reliable method by which isoimmunity to blood platelets can be detected. Technics for the quantitation of platelet survival by the use of radio-isotopically labeled platelets have been refined in the last few years and have been shown to possess a good degree of precision and reproducibility in animals as well as in humans.16-18
Various studies on the incidence of isoimmunity to blood platelets in multitransfused humans have been recently published. These were performed only by the use of in vitro methods and the degree of sensitivity of these serologic technics was never investigated by comparison with an in vivo method, that is, by determination of the survival of labeled donor platelets in the circulation of the multitransfused patients. This may explain why a relatively low incidence of isoimmunity to blood platelets in multitransfused patients was reported by the various investigators. Stefanini et al. found platelet agglutinins in 3 per cent of 295 multitransfused patients. Dausset et al., by the use of the same agglutinin technic applied in the rabbit studies presented here, reported an incidence of 13 per cent in 547 patients with a history of blood transfusions. Van De Wiel et al. found an incidence of platelet agglutinins of 5.4 per cent in 406 patients. By the use of the antihuman globulin consumption test, the same authors could detect platelet isosensitization in 14.3 per cent of the 406 patients. In the opinion of Van De Wiel et al., the latter test is more sensitive than the agglutinin and the complement fixation tests. Salmon and Schwartz, who studied isoimmunity to leukocytes and platelets in 639 multitransfused patients, obtained similar figures. However, when in our laboratory 10 normal volunteers were infused two to eight times with platelet concentrates obtained from 500 ml. of donor blood, in each instance isoimmunity to blood platelets was observed as demonstrated by reduced survival of Cr51-labeled homologous platelets infused in the recipients, while survival of the recipient's own platelets remained normal. Although these results obtained in a small number of healthy volunteers may not strictly imply that isoimmunity to blood platelets is to be expected in each patient who has received blood transfusions, they do, however, indicate that the occurrence of platelet isoantibodies in multitransfused patients is probably much more frequent than thus far reported and that the serologic technics available may be inadequate to detect in each instance the presence of these antibodies. Besides the lack of sensitivity of standard serologic technics, other reasons (“blocking” and other unusual types of antibodies) could also be invoked to explain their inadequacy in measuring platelet isoantibody in some sensitized patients. These have been discussed by Shulman et al. The results obtained in the present investigation in multitransfused rabbits are in support of this hypothesis. Furthermore, experiments which are still in progress in this laboratory, by the use of platelet survival and of simultaneous serologic studies in multitransfused patients, also indicate that the above hypothesis may be correct. A few experiments recently reported by Bosch et al. in humans again demonstrate a lack of correlation between reduction in the survival of homologous platelets in sensitized recipients and the results obtained with standard immunologic technics (Coombs' consumption test and Schultz-Dale reaction).

The fact that isoimmunity to blood platelets was obtained in most of the 17 multitransfused rabbits, either transfused with blood from one single donor or from multiple donors, is indicative of a multiplicity of iso-antigens in the blood platelets of this animal species. This conclusion was also reached
when in other studies\textsuperscript{15,24} it was demonstrated that rabbit blood platelets contain antigens in common with the skin and also histocompatibility antigens. Although the platelet antigenic structure in the rabbit may not be the same as in humans, the fact that similar results were obtained in 10 human volunteers who were sensitized by the repeated infusion of platelet concentrates\textsuperscript{19} may indicate that the same principle is valid also for human platelets. Grouping and typing of the blood platelets for the purpose of transfusion may therefore prove to be a very complex, if not an impossible task.

Two of our experiments in the rabbits demonstrated that isoimmunity to blood platelets tends to disappear when relatively long periods of time have elapsed after exposure to the antigenic stimulus. In one rabbit, however, a certain degree of reduction in survival of homologous platelets was still present 15 months after sensitization. Although the second set of studies was performed with platelets from donor animals which were different from the ones used soon after sensitization, the results are most probably significant since non-specific platelets were used in both experiments, immediately as well as 15 months after sensitization, and in the second set of studies platelets pooled from two donor rabbits were used. One experiment performed in a human volunteer in our laboratory gave a similar result and 8 months after sensitization the survival of homologous specific platelets was still markedly reduced to the same degree as found soon after sensitization. The long persistence of isoantibodies against platelet antigens in occasional patients has been pointed out also by others.\textsuperscript{13} Since the hemostatic property of transfused platelets depends greatly, if not entirely, on the capacity of the platelets to recirculate and survive in the recipient,\textsuperscript{21,22,24} these few observations may emphasize the serious consequences of platelet isoimmunity in those bleeding thrombocytopenic patients who are in need of prolonged platelet transfusion therapy.

**Summary**

The occurrence of platelet isoimmunity was studied in rabbits sensitized by 3 to 12 transfusions of homologous blood. Platelet isoimmunity in the transfused animals was detected and measured by two different technics: (1) determination of the survival of homologous platelets labeled in vivo with P\textsuperscript{32} and infused in the sensitized recipient rabbits; and (2) study of platelet agglutinins in the serum of the multitransfused animals.

It was found that:

1. After 3 to 12 blood transfusions, platelet sensitization, as determined by reduction in survival of homologous platelets in the transfused rabbits, occurred in most animals.
2. Survival of homologous specific platelets, i.e., platelets collected from the sensitizing donor and infused in the respective recipient, usually had lower values than the survival of homologous non-specific platelets.
3. Higher degrees of depression in survival of homologous platelets were found in the rabbits which had received larger numbers of blood transfusions.
4. Platelet isoagglutinins could be found only in three of the five rabbits.
which had been sensitized with 12 blood transfusions and the test was strongly positive in only one rabbit.

5. Repeat studies performed 2½ months after the transfusions demonstrated persistence of platelet iso-sensitization, while after 15 months iso-sensitization had greatly decreased; although in one rabbit a good degree of depression in homologous platelet survival was still present after this time interval.

These studies mainly demonstrate the high frequency of platelet iso-immunity in multitransfused rabbits and the inadequacy of the agglutinin test in the detection of even relatively severe degrees of platelet iso-sensitization.

**Summario in Interlingua**

Le occurrentia de isoimmunitate plachettal eseva studiate in conilios que habeva essite sensibilisate per medio de 3 a 12 transfusiones de sanguine homologe. Isoimmunitate plachettal in le animales post-transfusional eseva detegite e mesurate per duo differente technicas: (1) Determination del superviventia de homologe plachettas marcate in vivo con P³² e infusionate in le sensibilisate conilios, e (2) studio de agglutininas plachettal in le sero de multitransfusionate animales.

Esseva trovate:

1. Post 3 a 12 transfusiones de sanguine, sensibilisation plachettal—determinate per le reduction del superviventia de plachettas homologe in le conilios post-transfusional—occurreva in le majoritate del animales.

2. Le superviventia de homologe plachettas *specific*, i.e. plachettas colligite ab le sensibilisante donator e infusionate in le recipiente respective, eseva usualmente plus curte que le superviventia d homologe plachettas *non-specific*.

3. Plus alte grados de depression in le superviventia de homologe plachettas eseva trovate in le conilios que habeva recipite plus grande numeros de transfusiones de sanguine.

4. Isoagglutininas plachettal poteva esser trovate solmente in tres del cinque conilios que habeva essite sensibilisate con 12 transfusiones, e le test eseva fortemente positive in solmente un.

5. Studios repetitori, effectuate 2½ menses post le transfusiones demonstrava persistentia del iso-sensibilisation plachettal, sed post 15 menses, le iso-sensibilitate habeva decline grandemente. In un sol conilio un bon grado de depression del superviventia de homologe plachettas remaneva presente post iste intervallo de tempore.

Iste studios demonstra principalmente le alte frequentia de isoimmunitate plachettal in multitransfusionate conilios e le inadequatia del test de agglutinina in le detection de mesmo relativamente sever grados de iso-sensibilisation plachettal.

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