THE DETERMINATION OF THE LENGTH OF LIFE OF TRANSFUSED BLOOD CORPUSCLES IN MAN.

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PLATE 14.

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Whether transfused blood corpuscles live and function for any considerable length of time, or whether the beneficial results that have been observed to follow transfusion, outside the purely mechanical part of increasing the bulk of the depleted blood, are due to a stimulating effect on the hemopoietic function by the product of the broken down corpuscles is still an open question (Archibald, Crile, Hunter, Kimpton, Primrose, Robertson and Watson).

Marfels and Moleschott (1856) and Brown-Sequard (1867) studied the question of the length of life of blood corpuscles by injecting nucleated corpuscles (bird and frog) into animals having non-nucleated corpuscles, and watching for the disappearance of the nucleated corpuscles from the circulation of the transfused animal. It is now known that these results have no bearing on the question, as it was not blood destruction that was being studied but the elimination of foreign protein. The same objection holds good for any attempt to stain blood corpuscles with so called vital stain and to reinject them, since there is no stain known that will hold without destroying the living quality of the corpuscles; therefore, in the stained corpuscle we are again dealing with a foreign body capable of more or less rapid removal than transfused blood.

Thus far the only satisfactory direct methods of attacking the problem of the length of life of transfused corpuscles have been those involving the study of the changes of the erythrocyte count following transfusion. Extensive work along this line, done on animals by Ward-Muller, Quinck, von Ott, and Hunter, was reviewed by Hunter in 1885 and 1886. Ward-Muller transfused dogs and produced...
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a plethora. 2 or 3 days afterward he found that the number of blood corpuscles corresponded closely to the number of the original corpuscles plus the transfused; a few days later the injected corpuscles, judged by the decrease in blood count, began to break down, and by the end of a few weeks all the injected corpuscles had disappeared. In order to produce the effect of a very slow transfusion Hunter injected blood intraperitoneally and found that the blood count increased up to the 2nd or 3rd day, after which it gradually decreased and reached normal from the 14th to the 26th day, when 40 to 90 per cent of the total blood volume was injected. Von Ott, in order to avoid the abnormal condition of plethora, removed from one-half to two-thirds of the blood from animals and replaced it with defibrinated blood. He noted a gradual fall in the blood count which reached a minimum from the 19th to the 22nd day. From then on an increase took place which reached normal 2 weeks later. The lowest part of this curve would not, of course, represent the last of the transfused blood corpuscles but the point after which their destruction was surpassed by the regeneration of new corpuscles. The time that elapsed before the complete disappearance of transfused blood was something more than from 19 to 22 days, a result which agrees well with the findings of the workers previously mentioned.

These and similar results, although they argue strongly in favor of the prolonged life of the red blood corpuscles after transfusion, have not been accepted as proof thereof, because they depend on the blood count uncontrolled by the volume of the blood. It is argued that the prolonged high blood count after transfusion may not represent an increased total number of corpuscles but an upset of long duration in the blood-volume-controlling factors, which results in a decrease in the fluid content of the blood and a consequent increased cell count.

Since the satisfactory determination of changes in the blood volume offers difficulties at present unsurmounted, it would seem that any method that could finally settle the question of whether or not the transfused blood corpuscles have any lasting existence in the blood stream must depend on some means of identifying the transfused corpuscle without in any way changing it by stains, etc. It seemed possible to do this in man by making use of the blood groups that are so well defined there. In man there are four blood groups, based on the ability of the serum of one group to agglutinate the corpuscles of another (Sanford). The corpuscles of Group I are agglutinated by the serum of all other groups but its serum has no agglutinating properties. The serums of Groups II and III mutually agglutinate each other's
corpuscles. Group IV serum agglutinates all other corpuscles, while Group IV corpuscles are not agglutinated by any serum. It follows then that a person in Group I may receive the blood of a person of any other group, and that the blood of a person in Group IV may be given to a person belonging in any of the other groups, since danger in transfusing blood of an unlike group arises only when there is agglutination of the incoming corpuscles, the transfused serum being too much diluted when mixed with the recipient's blood to produce any agglutination of his own corpuscles. Such transfusions, especially from the Group IV donor into the Group I, II, or III recipient, are fairly commonly done in the Mayo Clinic. I have found that when a blood whose corpuscles are agglutinable is treated with a serum capable of agglutinating them, the agglutination may, by using sufficient serum, be made practically complete. The count of the few corpuscles lying between the clumps (Fig. 1) is usually from 0.5 to 0.7 per cent of the total blood count, but it may vary in different bloods from 0.03 to 3.4 per cent. When, however, unagglutinable corpuscles are mixed with agglutinable corpuscles, either by transfusion or in the test-tube before the agglutinating serum is added, there is a very large number of free corpuscles present (Fig. 2) which appear in the proportion in which the two kinds of corpuscles are mixed. Since we have a means of separating the corpuscles of two groups that have been mixed by agglutinating the corpuscles of one group and leaving the corpuscles of the other unagglutinated, and since it is possible to transfuse a patient with a group other than his own, we may, by taking samples of his blood from time to time after transfusion and differentially agglutinating his own corpuscles, tell from the abnormal number of unagglutinated corpuscles present how long the transfused blood remains in the circulation.

Technique.

Preliminary experiments were made with mixtures of bloods to determine the factors that it was necessary to control in order to obtain a technique which would give uniform results. Mixtures of corpuscles of known count were made; these were treated with the agglutinating serum, and after varying amounts of shaking, incubat-
ing, etc., a count was made of the unagglutinated corpuscles in a red blood cell counting chamber. In this preliminary procedure a series of mixtures of blood to serum 1:1, 1:5.5, 1:11, 1:22, and 1:55 was made. Although the 1:1 mixtures gave numerous unagglutinated corpuscles, 1:5.5 did not give a consistently greater percentage than any of the mixtures in which a greater proportion of serum was used; therefore, the 1:22 mixture, which was adopted because of its adaptability to use in the white cell counting pipette, should secure a maximum agglutination. Shaking during incubation probably frees some of the unagglutinable corpuscles that might otherwise be caught in the clumps of agglutinated corpuscles and thus increases the count of unagglutinated corpuscles in mixtures in which the unagglutinable corpuscles are present. Allowing the blood to stand in the ice box over night after incubation appears to give more uniform results. Slight differences in the length of time in the ice box caused no practical changes. Allowing the tubes to become warm after being in the ice box caused a decided increase in the count; thus it is advisable to keep each tube in the ice box until the count is to be made. Tubes may be thoroughly shaken to produce an even suspension before making a count without producing any increase in the unagglutinated corpuscles. 10 minutes shaking in a mechanical shaker with glass beads in the tube increases the count of the unagglutinated corpuscles only 1 to 2 per cent of the total blood count, and here heat rather than the shaking is probably the factor that causes the increase. If the unagglutinated corpuscles are so thick that the count cannot readily be made, it is possible to make a dilution with cold serum. It is not advisable to use normal salt solution as that increases the count. Partly as a result of this preliminary work and partly for the sake of uniformity, the following technique was adopted.

Blood is taken from the ear in a white cell counting pipette to the 0.5 mark, the pipette is then filled to the 11 mark with the agglutinating serum to which a 4.4 per cent citrate solution has been mixed in the proportion of 20 : 1, and the whole is expelled into a small test-tube and shaken, thus giving a 1 : 22 mixture of blood and citrated serum. This mixture is incubated at 37°C. for 40 minutes with thorough shaking every 10 minutes and is left in the ice box over night. The mixture is then thoroughly shaken and a drop of it is placed in a
red blood cell counting chamber and a count of the unagglutinated corpuscles is made. 160 squares in each of two chambers are counted, the average is taken, and the count multiplied by \(\frac{1,100}{2}\) to give the number of unagglutinated corpuscles per cubic millimeter of blood. A tube containing the blood of a person who has not been transfused is always used to control the effectiveness of the serum, and serum from the same person is used for agglutinating throughout a set of experiments.

**Differential Agglutination in Vitro.**

*In vitro* experiments were done to establish whether or not the results obtained by agglutinating the agglutinable corpuscles in a mixture of unagglutinable and agglutinable corpuscles were quantitative, and to determine whether, in such a mixture, the number of unagglutinable corpuscles counted is equal to the number actually present or whether it is some function of that number. To show the accuracy of the technique under absolutely uniform conditions with the same blood and serum and simultaneous shaking and incubation, two tests were made of each of a series of mixtures with different pipettes and the results compared (Table I).

These figures show a degree of accordance between each of two distinct tests which leaves no doubt as to their quantitative character.

In order to compare the number of unagglutinated corpuscles with the known number of agglutinable corpuscles present, mixtures of a Group IV blood with a Group II blood were made by the drop method in the proportions of 1:30, 1:15, 1:10, etc.; the same capillary pipettes were used throughout. Samples of these mixtures, which had been thoroughly shaken, were diluted in the white cell counting pipette with the Group IV serum, after which the usual technique was followed. Two agglutinations were made from each mixture and the count of the unagglutinated cells was averaged after subtracting the number of unagglutinated corpuscles found in the pure Group II blood, which in this instance was rather high, being 119,900 per c.mm. The number thus obtained was compared with the number of the Group IV corpuscles computed to be present, and found by dividing
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the count of the Group IV blood used by the fraction which it composed of the whole mixture. The results are shown in Table II.

Through a wide range of mixtures the counted number of unagglutinated corpuscles approximated closely the number of unagglutinable Group IV corpuscles computed to be present.

<table>
<thead>
<tr>
<th></th>
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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1 : 30</td>
<td>4,880,000</td>
<td>235,400</td>
<td>209,000</td>
<td>26,400</td>
<td>0.58</td>
</tr>
<tr>
<td>1 : 25</td>
<td>4,870,000</td>
<td>249,700</td>
<td>228,800</td>
<td>10,900</td>
<td>0.22</td>
</tr>
<tr>
<td>1 : 20</td>
<td>4,810,000</td>
<td>335,500</td>
<td>325,600</td>
<td>9,900</td>
<td>0.20</td>
</tr>
<tr>
<td>1 : 15</td>
<td>4,800,000</td>
<td>474,200</td>
<td>464,200</td>
<td>6,000</td>
<td>0.14</td>
</tr>
<tr>
<td>1 : 10</td>
<td>4,710,000</td>
<td>578,600</td>
<td>566,300</td>
<td>12,100</td>
<td>0.26</td>
</tr>
<tr>
<td>1 : 5</td>
<td>4,500,000</td>
<td>894,300</td>
<td>887,700</td>
<td>6,600</td>
<td>0.15</td>
</tr>
</tbody>
</table>

Average percentage of error........................................ 0.25

<table>
<thead>
<tr>
<th>Proportion of Group IV corpuscles mixed with Group II corpuscles.</th>
<th>Unagglutinated corpuscles per c. mm. minus 119,900.</th>
<th>Calculated No. of Group IV corpuscles per c. mm.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 : 30</td>
<td>127,370</td>
<td>137,000</td>
</tr>
<tr>
<td>1 : 15</td>
<td>228,570</td>
<td>267,000</td>
</tr>
<tr>
<td>1 : 10</td>
<td>391,600</td>
<td>388,100</td>
</tr>
<tr>
<td>1 : 6</td>
<td>572,773</td>
<td>610,000</td>
</tr>
<tr>
<td>1 : 4</td>
<td>803,559</td>
<td>854,000</td>
</tr>
<tr>
<td>1 : 3</td>
<td>1,085,320</td>
<td>1,037,500</td>
</tr>
</tbody>
</table>

Evidence that the Unagglutinated Corpuscles Observed after Transfusion Are the Transfused Corpuscles.

Before the abnormal increase of unagglutinated corpuscles found in the blood of Group I, II, or III patients transfused with Group IV blood could be assumed to be due to the presence of the transfused blood, it was considered necessary to run control experiments covering two points: (1) to see that this increase in count of unagglutinated
corpuscles does not appear when a patient with blood of an agglutinable group is transfused with blood of the same group, and, therefore, that the appearance of unagglutinated corpuscles is not some non-specific reaction incident to the introduction of foreign blood; (2) to see that the appearance of the unagglutinable corpuscles is not due to a reaction of the transfused Group IV serum on the recipient's native corpuscles.

It was also of interest to note, in as far as it was practicable with our insufficient knowledge of the blood volume, whether there was any correlation between the amount of blood transfused and the number of unagglutinated corpuscles which appear in the circulation following transfusion with unagglutinable blood. That the increase in unagglutinable corpuscles does not appear except when unagglutinable blood is transfused is shown in the following results.

Of four patients who were transfused with blood of a like group, one gave 91,300 per c.mm. unagglutinated corpuscles before transfusion and 42,900 per c.mm. after transfusion, when 200 cc. of blood were transfused. In the three other cases, when 500 cc. were transfused, 51,700, 52,800, and 8,800 per c.mm. of unagglutinated corpuscles respectively were counted. As the agglutination with no transfusion has varied within the limits of from 0.03 to 3.4 per cent of the blood count, these figures are well within normal limits.

On the assumption that the appearance of unagglutinated corpuscles might be due to the transfused serum, the following experiment was done. During a transfusion on a Group II patient which lasted less than 30 minutes and in which 500 cc. of citrated Group IV blood were transfused, samples of blood were taken from the ear, one after 300 cc. and one after 500 cc. of blood had been given. A control had been taken immediately before the transfusion. The results obtained by agglutinating with Group IV serum were: before transfusion 41,690 unagglutinated corpuscles per c.mm., after transfusion of 300 cc. 269,000 unagglutinated corpuscles per c.mm., and after transfusion of 500 cc. 478,390 unagglutinated corpuscles per c.mm. Samples of blood taken before transfusion, both citrated and uncitrated, in this case and in others gave no increase in the unagglutinated corpuscle count when mixed with Group IV serum in the proportion of 1 : 22 and incubated for 24 hours. It would seem that if in vitro Group IV
serum does not affect the agglutinability of Group II corpuscles during 24 hours at body temperature in over 100 times the concentration in which it appears in the recipient's body, the immediate increase in unagglutinable corpuscles obtained during transfusion is not due to a reaction of any constituents of the transfused serum on the recipient's native corpuscles.

Whether or not the unagglutinated corpuscles appear in numbers proportional to the amount of blood transfused is shown in seven patients, of whom six were transfused with Group IV blood and one was a Group I patient who was transfused with Group III blood. The count of the unagglutinated corpuscles was compared with a computed figure obtained from the amount of blood transfused, the body weight, and a factor common to the series. If the correction is omitted which would be necessary if differences exist in different persons in the proportion between blood and body weight, concerning which unfortunately we have no satisfactory data, it may be assumed that the number of unagglutinated corpuscles which appear should be directly as the amount of blood transfused and inversely as the body weight. Taking \( x \) as the common unknown factor in Case A (Table III), in which the amount of blood transfused was 200 cc., the body weight 133.5, and the count of unagglutinated corpuscles 220,000, we have

\[
220,000 = \frac{200}{133.5} \cdot x
\]

Then by solving for \( x \) whose value is 146,850 and substituting in a similar equation in Case B in which the amount of blood transfused was 500 cc. and the body weight 151 pounds, a computed figure will be obtained, which may be called \( y \), for the number of Group IV corpuscles which should be present per c.mm. Thus

\[
y = \frac{500}{151} \times 146,850
\]

\[y = 486,258\]

If we continue this process through the series, computed figures are obtained which give a basis of comparison of the results obtained in different cases. A comparison of these computed figures, with the
counted number of corpuscles, is given in Table III. If due allowance is made for the inherent factor of error in the computed figures it will be seen that the degree of agreement between these two sets of figures indicates a quantitative relation between the amount of blood transfused and the number of unagglutinated corpuscles which appear in the circulation after transfusion. The weight at the time of transfusion was used in all instances except in Case B who could not be weighed.

**TABLE III.**

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Reason for transfusion.</th>
<th>Weight</th>
<th>Amount of blood transfused,</th>
<th>Calculated No. of Group IV corpuscles per c. mm.</th>
<th>Counted unagglutinated corpuscles per c. mm.</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Preparatory to operation.</td>
<td>133.5</td>
<td>200</td>
<td>486,258</td>
<td>220,000</td>
</tr>
<tr>
<td>B</td>
<td>Pernicious anemia. Transfused 20 days previously with 500 cc. of Group IV blood.</td>
<td>151</td>
<td>500</td>
<td>764,843</td>
<td>551,000</td>
</tr>
<tr>
<td>C</td>
<td>Hemorrhage following Kraske operation.</td>
<td>96</td>
<td>500</td>
<td>764,843</td>
<td>698,000</td>
</tr>
<tr>
<td>D</td>
<td>Pernicious anemia. Transfused 19 days previously with Group IV blood. The initial count of unagglutinated corpuscles was 10 per cent of the blood count.</td>
<td>96</td>
<td>500</td>
<td>764,843</td>
<td>980,000</td>
</tr>
<tr>
<td>E</td>
<td>Anemia.</td>
<td>135</td>
<td>500</td>
<td>543,888</td>
<td>700,000 (minus initial count).</td>
</tr>
<tr>
<td>F</td>
<td>Hemorrhage following hysterectomy.</td>
<td>138</td>
<td>1,200</td>
<td>1,276,956</td>
<td>1,412,400</td>
</tr>
<tr>
<td>G</td>
<td>Anemia (infant).</td>
<td>20.5</td>
<td>250</td>
<td>1,790,853</td>
<td>1,175,900</td>
</tr>
</tbody>
</table>

_Evidence of the Prolonged Existence of Transfused Blood Corpuscles._

Application of this technique was undertaken for the purpose of determining whether or not transfused blood lives in the circulation, and specimens of blood of patients of an agglutinable group transfused with Group IV blood were taken before, and at short intervals after transfusion. These specimens were examined for the persistence after transfusion of abnormal numbers of unagglutinated cor-
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puscles. The protocols of Cases G, E, and F give the counts of un-agglutinated corpuscles and a simultaneous study of the total blood count (Text-fig. 1). These figures represent the average of two pipettes. The unagglutinated corpuscles of the normal untransfused Group II individual who was used as a control varied through the experiments from 0.5 to 0.7 per cent of the total blood count.

Text-Fig. 1. The rate of change in the number of transfused corpuscles per cubic millimeter and the accompanying changes in the total blood count.

Case G.—Fat infant, age 17 months; weight 20.5 pounds. Belongs to Group II; had a low blood count. A diagnosis of secondary anemia had been made Sept. 20, 1918. The red blood cells were 1,660,000; the unagglutinated corpuscles were 54,400 per c.mm. A transfusion was done of 250 cc. of citrated Group IV blood.
Sept. 21. Red blood corpuscles 2,375,000; unagglutinated corpuscles 1,175,000 per c.mm.
23. " 2,380,000; " 1,192,400 " "
25. " 2,260,000.
29. " 2,570,000; unagglutinated corpuscles 932,900 per c.mm.
Oct. 2. " 2,280,000; " 602,250 " 
4. Sent to the hospital with an attack of influenza.
7. Died.

Case E.—A Group II individual, age 32 years. Had a secondary type of anemia, possibly from hemorrhage due to piles. The patient’s normal weight was 160 pounds, the present weight was 135 pounds. Oct. 1, 1918. The red blood cells were 2,100,000; the unagglutinated cells were 1,100 per c.mm.
A transfusion was done with 500 cc. of Group IV blood.

Oct. 12. Red blood corpuscles 2,820,000; unagglutinated corpuscles 409,200 per c.mm.
15. Unagglutinated corpuscles 318,000 per c.mm.
17. Red blood corpuscles 2,705,000; " 390,700 " 
Mild attack of influenza.
21. Red blood corpuscles 2,600,000; unagglutinated corpuscles 333,700 per c.mm.
24. " 2,660,000; " 367,400 " 
25. Went home for 2 weeks.

Nov. 9. Red blood corpuscles 2,510,000; unagglutinated corpuscles 251,020 per c.mm.
10. " 260,700 " 
12. Red blood corpuscles 2,105,000; " 258,720 " 
13. " 2,185,000; " 245,070 " 
A transfusion was done of 500 cc. of Group IV blood.
14. Red blood corpuscles 2,625,000; unagglutinated corpuscles 585,200 per c.mm.
20. " 2,760,000; " 517,000 " 

Case F.—Age 35 years; normal weight 140 pounds; present weight 138. Operated on for fibroid of the uterus. Sept. 21, 1918. The unagglutinated cells were 88,000 per c.mm.; the blood count could not be obtained. 700 cc. of Group IV blood were given because of severe hemorrhage; the hemorrhage continued and an additional 500 cc. of Group IV were given.

Sept. 23. Red blood corpuscles 3,800,000; unagglutinated corpuscles 1,412,400 per c.mm.
25. " 3,729,000; " 807,400 " 
27. " 4,099,000; " 1,139,800 " 
30. " 1,293,800 " 

Oct. 2. Red blood corpuscles 4,920,000; " 590,700 " 
4. " 4,405,000; " 704,000 " 
7. " 4,000,000; " 870,100 " 
10. " 4,060,000; " 737,000 " 
12. " 4,250,000; " 702,900 " 
15. " 796,400 " 
19. " 4,550,000; " 299,200 " 
25. " 4,109,000; " 118,800 " 
30. " 4,302,000; " 51,700 " 
The greater irregularities in the count of unagglutinated corpuscles in this protocol are due to the fact that the patient's blood often clotted the Group IV serum with which it was mixed. This tendency was overcome later by increasing the strength of the citrate solution from 2 per cent to 4.4 per cent.

It will be noted in these cases that in each instance before transfusion there was an initial count of unagglutinated corpuscles which came within the limits of the counts found in other untransfused persons, while after transfusion there was a very great increase in unagglutinated corpuscles. In Case F, a patient who had had a severe hemorrhage and in whom the amount of blood transfused was great, this increase amounted to over 30 per cent of the total blood count; in Case G in whom the initial blood count was extremely low and the amount of blood given in proportion to the body weight was also great, it amounted to nearly 50 per cent of the patient's blood count. The increase was maintained for a long time with a surprisingly slow fall. In Case G who died 5 days after the last count was taken, only half the number of unagglutinated corpuscles which appeared after transfusion had disappeared in 12 days; in Case E whose condition remained on a level notwithstanding a slight attack of grippe, there was no marked decrease in the original number of unagglutinable corpuscles after 13 days, and in 33 days there was only a 37 per cent fall; in Case F, a comparatively normal patient who made an excellent recovery from operation, there was a marked decrease in unagglutinated corpuscles between the 25th and 28th days, and the agglutinability of the patient's blood may be considered to have approximately reached normal by the 30th day. In all cases the unagglutinated corpuscles, as long as they were present in considerable numbers, appeared to be in good condition.

I have shown that this differential agglutination reaction is a constant and quantitative one and that the unagglutinated corpuscles are present in numbers equal to the unagglutinable corpuscles admixed, so that unless something takes place in the body that does not take place in the test-tube, the counts of unagglutinated corpuscles after transfusion may be assumed to be a quantitative indicator of the number of transfused corpuscles present. As the unagglutinated corpuscles appear after transfusion in numbers proportionate to the amount of unagglutinable blood transfused, as they do not appear
when agglutinable blood is transfused, and, moreover, as they are not caused by any effect of the Group IV serum on the recipient’s corpuscles, it seems safe to assume that these unagglutinated corpuscles are the transfused corpuscles and that their number is a quantitative indicator of the amount of transfused blood present in the recipient’s circulation. It must follow then that the prolonged appearance of unagglutinated corpuscles in the blood stream of these transfused patients can only mean that the transfused corpuscles existed for a long time in the circulation, and it seems that they not only existed but functioned.

In considering these results, the question of to what extent they apply to the more usual condition when the patient is transfused with blood of his own group, immediately arises. Since the peculiarities which are brought out in blood grouping are probably peculiarities which will also be found in all the cells of the body, we may be able to turn to other tissues for light on this point. In this connection Masson’s work in skin grafting is of interest.

"In all patients requiring skin grafting who were under my care during the past year, the blood of the donor, as well as that of the recipient, has been tested for agglutination. The results have been very interesting and instructive, and I feel sure will add a great deal to the popularity of the use of the isograft. . . . . I have tested the principle with the three varieties of grafts, and am satisfied that blood grouping is just as important for good results in skin grafting as it is necessary in transfusion, and that it is governed by the same principles. While the results are not positive, nevertheless, I have never had a skin take which was removed from a donor whose red blood corpuscles were agglutinated by the serum of the patient. The results in all other cases have been very satisfactory, almost, if not entirely, equal to autodermic grafting."

It would seem probable, if further investigation bears out the above results with skin grafts, that the results obtained in studying the length of life of transfused Group IV corpuscles in a recipient of another group hold for the life of the corpuscles of any group transfused into the recipient of a like group, and that it is even probable that these results hold for the length of life of the native corpuscle.

Since my results show that there is no immediate breaking down of transfused cells which could cause a stimulation of the bone marrow, and since it is more probable, as suggested by Lindeman, that when
there is a stimulation of the bone marrow following transfusion it is
due to improved metabolic conditions of the bone marrow cells,
brought about by the larger number of corpuscles in the blood; it would
seem, on the surface, that the logical procedure in transfusion would
be to push the transfusions until a normal blood count was attained,
making the time interval between transfusions only long enough to
allow the fluid content to establish itself. This would be indicated
by the rise in the blood count which takes place a day or two after
transfusion.

CONCLUSIONS.

1. It is possible in mixtures of corpuscles of different groups to
separate the corpuscles practically quantitatively by treating with a
serum that agglutinates the corpuscles of one kind, leaving the
others unagglutinated.

2. After a recipient has been transfused with blood of a group
other than his own, specimens of his blood treated with a serum
that will agglutinate his own corpuscles but not the transfused
corpuscles show unagglutinated corpuscles in large numbers.

3. These unagglutinated corpuscles which appear in the recipient’s
blood after such a transfusion are the transfused corpuscles and
their count is a quantitative indicator of the amount of transfused
blood still in the recipient’s circulation.

4. The life of the transfused corpuscle is long; it has been found to
extend for 30 days and more. The beneficial results of transfusion
are without doubt not due primarily to a stimulating effect on the
bone marrow, but, it is reasonable to assume, to the functioning of the
transfused blood corpuscles.

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von Ott, quoted by Hunter.


Quinck, quoted by Hunter.


Ward-Muller, quoted by Hunter.

**EXPLANATION OF PLATE 14.**

**Fig. 1.** Microphotograph of the agglutinated blood of a person not transfused.

**Fig. 2.** Microphotograph showing the clumps of agglutinated native corpuscles and the unagglutinated transfused corpuscles of blood of an individual 16 days after transfusion.
Fig. 1.

Fig. 2.

(Ashby: Transfused blood corpuscles.)