Transfusion Reactions due to Plasma Incompatibility in Dogs

By J. Q. Bliss, M.D., D. G. Johns, M.D., and A. S. V. Burgen, M.B., M.R.C.P.

Plasma incompatibility was investigated as a possible factor in transfusion reactions in dogs. Urticaria, failure to retain within the circulation the equivalent of transfused plasma volume and protein, and increased gastric acid secretion almost invariably followed transfusion of plasma from another dog but not transfusion of the dog's own plasma. The cutaneous reactions were abolished and the retention of plasma was improved by administration of an antihistamine. Erythrocyte isoagglutinins did not appear to be involved in these reactions.

Methods

Mongrel dogs weighing 20 to 30 Kg. were used for all experiments except those under anesthesia, which were smaller (5 to 10 Kg.). Fewer blood samples were taken from the smaller dogs without undue interference with the blood volume. None of the animals had previously received any dog plasma or other injections.

Plasma was obtained by a two-stage bleeding on consecutive days from a lateral leg vein exposed under local anesthesia. A free flow of blood was ensured by introducing a polyethylene catheter as far as the inferior vena cava. Twenty ml. of blood/Kg. weight were collected on each occasion into heparin (100 mg./L.). On no occasion did signs of shock ensue. The blood was centrifuged at 2,000 r.p.m. (r = 20 cm.) for 10 min., the plasma aspirated off, and the cells, resuspended in isotonic saline, were reinfused into the animal. Chloramphenicol, 20 mg./L., was added to the plasma, which was stored at a temperature of 4 C. It was never kept longer than 48 hours and was always recentrifuged immediately before use.

Transfusions of plasma were made at a controlled rate through a catheterized leg vein. Circulating plasma volumes were measured with T 1824, the concentration at zero time being determined by extrapolation back from the concentration of 4 samples taken at 7.5 min. intervals after the injection of the dye. Dye concentrations were measured after acetone extraction. Plasma protein concentration was determined by a biret method as modified by Stewart and Burgen, plasma hemoglobin by the method of Flink and Watson, and the hematocrit in duplicate by centrifugation at 2,500 r.p.m. for 30 min.

Plasma and serum samples were examined for erythrocyte isoagglutinins by cross matching in saline and in concentrated albumin solution.

An assessment of the sensitivity of the dog to the donor plasma was made by intradermal injec-

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TABLE 1.—Effect of the Bleeding Procedure on the Plasma Volumes, Cell Volumes, and Total Plasma Proteins of 8 Normal Dogs

<table>
<thead>
<tr>
<th></th>
<th>Prebleed</th>
<th>Pretransfusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma volume (ml./Kg.)</td>
<td>48.5 ± 1.9†</td>
<td>44.8 ± 1.2 (p &lt; 0.02)†</td>
</tr>
<tr>
<td>Cell volume (ml./Kg.)</td>
<td>34.5 ± 1.5</td>
<td>33.5 ± 1.7 (p = 0.3)†</td>
</tr>
<tr>
<td>Plasma protein (Gm./Kg.)</td>
<td>3.25 ± 0.12</td>
<td>2.71 ± 0.09 (p &lt; 0.001)‡</td>
</tr>
</tbody>
</table>

*Two procedures per dog, spaced 2 weeks apart.
†Mean ± standard error.
‡Value for t, prebleed vs. pretransfusion.

To our knowledge the source of the plasma was the only variable in this experiment. The recipients of both autologous and nonautologous transfusion had undergone identical bleeding schedules and were entirely comparable in initial blood volume and hematocrit. The samples of autologous and nonautologous plasma were obtained, stored, and administered in the same way.

In table 1 the values for plasma volume, red cell volume, and plasma protein are given for the animals before bleeding and before transfusion. The plasma volume was reduced on the average by 7.6 per cent and the circulating plasma protein by 16.6 per cent as a result of the bleeding. However, these reductions were small relative to the volume and plasma protein content of the subsequent transfusions.

Within 15 min. after transfusion of nonautologous plasma every animal developed cutaneous whealing, particularly in the neck and groin regions. The wheals were bright blue in color owing to the leakage of plasma stained with T-1824 and were preceded and surrounded by erythema. In the more severe reactions the wheals were generalized and confluent, and facial edema occurred, particularly in the muzzle and periorbital tissues. The severe reactions were accompanied in some animals by vomiting and lethargy. On the other hand, no cutaneous or other reactions were seen after any of the autologous transfusions.

Table 2 gives the values for changes in plasma volume, protein, and red cell volume following transfusion. Retention of transfused volume within the circulation after autologous plasma was complete, but after nonautologous plasma only 51.5 ± 10.3 per cent of the transfused volume remained in the circulation 30 min. after the transfusion. No significant further loss occurred during the subsequent 30 min. The estimated amount of red cell volumes remained so nearly constant throughout the experiments that the change in hematocrit provided almost as accurate a measure of the degree of plasma expansion as did the dye dilution method.
### Table 2.—Effect of Autologous and Nonautologous Plasma Transfusions on the Plasma Volumes, Red Cell Volumes, and Total Plasma Protein of 8 Normal Dogs

<table>
<thead>
<tr>
<th></th>
<th>Autologous</th>
<th>Nonautologous</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Pretransfusion plasma volume (ml./Kg.)</strong></td>
<td>45.0 ± 1.5*</td>
<td>45.0 ± 2.0</td>
</tr>
<tr>
<td>60 min. before</td>
<td>44.2 ± 1.8</td>
<td>44.6 ± 2.1</td>
</tr>
<tr>
<td>30 min. before</td>
<td>65.7 ± 2.1</td>
<td>55.3 ± 3.0</td>
</tr>
<tr>
<td>30 min. after</td>
<td>65.2 ± 2.6</td>
<td>54.9 ± 3.1</td>
</tr>
<tr>
<td>60 min. after</td>
<td>104.0 ± 8.9</td>
<td>51.5 ± 10.3</td>
</tr>
<tr>
<td>**Plasma volume expansion† (as percentage of volume transfused)</td>
<td>126.1 ± 10.8</td>
<td>58.0 ± 10.1</td>
</tr>
<tr>
<td>45 min. after</td>
<td>99.8 ± 4.3</td>
<td>99.5 ± 6.6</td>
</tr>
<tr>
<td>**Plasma protein expansion† (as percentage of amount transfused)</td>
<td>104.0 ± 8.9</td>
<td>51.5 ± 10.3</td>
</tr>
<tr>
<td>45 min. after</td>
<td>126.1 ± 10.8</td>
<td>58.0 ± 10.1</td>
</tr>
<tr>
<td><strong>Red cell volume† (as percentage of pretransfusion red cell volume)</strong></td>
<td>99.8 ± 4.3</td>
<td>99.5 ± 6.6</td>
</tr>
<tr>
<td>45 min. after</td>
<td>104.0 ± 8.9</td>
<td>51.5 ± 10.3</td>
</tr>
</tbody>
</table>

*Mean ± standard error.
†Value for t, autologous vs. nonautologous.
‡Averaged for each animal from 30 and 60 min. pretransfusion and post-transfusion determinations.

After the nonautologous transfusions, much of the equivalent of the transfused plasma protein was lost from the circulation, but with autologous transfusions the increase in circulating proteins appeared to be slightly greater than that transfused.

It is, of course, of great interest to know whether the intensity of the intradermal reaction to plasma reflects the degree of whealing and of deficient retention of transfused plasma. In table 3 are summarized the results in 16 dogs, the 8 dogs of group 1 and the 8 control animals receiving nonautologous transfusions in group 2. There is a fairly good correlation between the severity of whealing and the titer of the intradermal reaction. The correlation of either of these with retention of transfused plasma is poor.

**Group 2. Effect of Mepyramine on the Nonautologous Plasma Reactions.** In view of the previous demonstration that the whealing produced by the intracutaneous injection of nonautologous plasma could be prevented by an antihistamine, an experiment was carried out to determine whether the reactions to transfused nonautologous plasma could be similarly prevented in whole or in part. Mepyramine maleate (Neoantergan) was selected because of its high degree of specificity. Plasma was obtained by the standard bleeding procedure from 8 pairs of dogs of matched weights. Each member of a pair served as donor for the other, so that all animals received only nonautologous transfusions. Twenty ml./Kg. plasma were transfused at the rate of 1 ml./Kg./min. No anesthesia was used. One member of each pair received intravenously 5 mg./Kg. mepyramine as a 1.5 per cent solution in divided doses 30 and 15 min. before transfusion. The other member served as a control.

None of the animals treated with mepyramine developed wheals, whereas 7 of the 8 untreated animals developed wheals similar to those of the previous experiment. Table 4 shows that expansion of plasma volume and retention of plasma protein were considerably greater in the treated animals than in the untreated, but still were significantly less than the complete expansion found after autologous transfusion (p < 0.02 for both volume and protein expansion). In 3 supplementary control experiments, mepyramine alone did not affect the plasma volume or circulating protein.

**Group 3. Effects of Transfusions on Blood Pressure and Gastric Acidity.** Eight dogs under anesthesia were transfused with 20 ml./Kg. of plasma at a rate of 2ml./Kg./min. Four animals were given autologous, and the
TABLE 3.—Intradermal Titer, Whealing Reaction, and Plasma Expansion in Dogs Receiving Nonautologous Plasma Transfusions

<table>
<thead>
<tr>
<th>Pretransfusion intradermal titer*</th>
<th>Whealing after transfusion†</th>
<th>Plasma volume expansion, 45 min. after transfusion (as percentage of volume transfused)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/27</td>
<td>4.0‡</td>
<td>19%‡</td>
</tr>
<tr>
<td></td>
<td>(4, 4, 4)</td>
<td>(33, 16, 9)</td>
</tr>
<tr>
<td>1/9</td>
<td>2.7</td>
<td>51%</td>
</tr>
<tr>
<td></td>
<td>(3, 3, 2)</td>
<td>(56, 19, 78)</td>
</tr>
<tr>
<td>1/3</td>
<td>2.1</td>
<td>58%</td>
</tr>
<tr>
<td></td>
<td>(2, 3, 1, 5, 3, 3)</td>
<td>(26, 70, 60, 71, 2, 3, 3)</td>
</tr>
<tr>
<td>Undiluted</td>
<td>1.4</td>
<td>47%</td>
</tr>
<tr>
<td></td>
<td>(4, 3, 1, 5, 0)</td>
<td>(13, 66, 93, 16)</td>
</tr>
</tbody>
</table>

*Highest dilution producing a detectable response.
†Graded on an arbitrary scale: 0, no whealing; 1, one or two wheals; 2 and 3, moderate whealing; 4, confluent whealing with severe facial edema.
‡Mean value. Individual values in brackets.

other 4 nonautologous, plasma. Again, the animals of both series were subjected to the same bleeding procedure, and both types of plasma were obtained and treated in the same way. The recipients of nonautologous plasma, however, were deliberately selected on the basis of their skin responses to intradermally injected donor plasma: only those which showed reactions to a dilution of 1/9 or higher were used.

The 4 animals receiving autologous plasma showed slight pressor responses. Three of the 4 dogs receiving nonautologous transfusions showed a similar response, but the fourth animal developed a severe hypotension, the mean blood pressure falling from 115 mm. Hg to 40 mm. Hg near the end of the transfusion, and then remaining low for almost an hour. Similar hypotensive responses have been observed in several pilot experiments.

A marked increase in gastric hydrochloric acid secretion was seen after all 4 nonautologous transfusions (fig. 1) but no such response was seen after autologous transfusion.

Erythrocyte Isoagglutinins. Immediately before 10 of the nonautologous transfusions the sera of both donor and recipient were tested for erythrocyte isoagglutinins. None was detected. Further evidence that the nonautologous effect is unrelated to any erythrocyte factor was obtained from 2 pairs of dogs which had shown strong reactions to each other's plasma. Four weeks after plasma transfusion these dogs were transfused with 100 ml. of each other's washed erythrocytes, suspended in 100 ml. of isotonic saline. No signs of whealing, collapse, or other untoward reaction occurred, and plasma hemoglobin determinations taken before, and at hourly intervals for 6 hours after, transfusion gave no evidence of a hemolytic reaction.

DISCUSSION

These results show that in the unanesthetized dog relatively large transfusions of nonautologous plasma almost always produce minor to moderate reactions and occasionally produce major reactions of an anaphylactoid type. Although urticaria is the commonest manifestation of these reactions, a more significant feature is a loss of the equivalent of much of the added plasma within 30 min. of transfusion. This loss is, on the average, sufficient to reduce the increment in plasma volume to about half the predicted value; occasionally it is so pronounced that the plasma volume is not expanded at all.

The efficiency of autologous plasma as a plasma volume expander, as well as the absence of reactions following its use, provides valuable evidence that the reactions we have described are not due to the methods of handling or storing the plasma but to a genuine incompatibility. We can find no evidence to connect this incompatibility with the presence of natural erythrocyte isoagglutinins in the plasma. The occurrence of these isoagglutinins has been reported to be infrequent,\textsuperscript{12} and in our own experiments we have been unable to demonstrate either natural isoagglutinins or untoward reactions to red cell infusions.

The reactions to nonautologous plasma transfusions appear to be the result of a release of endogenous histamine: the urticarial reaction is completely inhibited by pretreatment with an antihistamine, and a sharp increase in gastric acid secretion often follows transfusion. Facial edema and fatal circu-
TABLE 4.—Effect of Nonautologous Plasma Transfusions on the Plasma Volumes, Red Cell Volumes, and Total Plasma Protein of 8 Normal Dogs and of 8 Dogs Treated with Mepyramine Maleate

<table>
<thead>
<tr>
<th></th>
<th>Treated</th>
<th>Untreated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pretransfusion plasma volume (ml./Kg.)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>80 min. before</td>
<td>50.4 ± 3.1*</td>
<td>48.0 ± 2.5</td>
</tr>
<tr>
<td>50 min. before</td>
<td>50.6 ± 2.9</td>
<td>47.0 ± 2.6</td>
</tr>
<tr>
<td>Post-transfusion plasma volume (ml./Kg.)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>30 min. after</td>
<td>65.9 ± 3.5</td>
<td>56.8 ± 3.3</td>
</tr>
<tr>
<td>60 min. after</td>
<td>63.7 ± 3.6</td>
<td>55.0 ± 3.4</td>
</tr>
<tr>
<td>Plasma volume expansion†(as percentage of volume transfused)</td>
<td>71.5 ± 7.8</td>
<td>40.5 ± 8.9 (p &lt; 0.03)‡</td>
</tr>
<tr>
<td>Plasma protein expansion†(as percentage of amount transfused)</td>
<td>88.8 ± 9.4</td>
<td>45.2 ± 10.6 (p &lt; 0.02)‡</td>
</tr>
<tr>
<td>Red cell volume† (as percentage of pretransfusion red cell volume)</td>
<td>106.0 ± 2.6</td>
<td>108.1 ± 8.7</td>
</tr>
</tbody>
</table>

*Mean ± standard error.
†Averaged for each animal from 80 and 50 min. pretransfusion and 30 and 60 min. posttransfusion determinations.
‡Value for t, treated vs. untreated.

Laboratory collapse occur after nonautologous transfusions in the adrenalectomized dog and can be prevented by the previous administration of the antihistaminic drug, diphenhydramine. These results are concordant with the evidence for mediation by histamine found after intradermal injection of nonautologous plasma. The failure of an antihistamine to completely prevent plasma extravasation while it prevents the urticaria is not surprising, in view of the known resistance to the antihistamines of some effects of the histamine liberators. Since skin lesions or bluing no longer occur after treatment with an antihistamine, the observed loss of plasma under these conditions is presumably occurring into tissues other than the skin.

Although skin testing is the easiest and most dramatic method of showing the plasma incompatibility, it is not a method which readily lends itself to statistical analysis, because of the difficulties in objective assessment of both skin responses and transfusion reactions. Also, it is probable that the skin sensitivity to donor plasma is only one of several factors determining the severity of the reaction to transfused plasma (for example, the initial rate at which nonautologous protein reaches the extravascular space and the tissue mast cells). The results of skin testing do suggest, however, that animals which show marked skin sensitivity to donor plasma also have severe reactions to transfusions of that plasma. Animals which show weak skin responses react to transfusion in unpredictable ways.

Although few of our transfusions were performed under anesthesia, we gained the impression that whealing was less prominent than it was in conscious animals. Similarly, facial edema has been reported to be less prominent after transfusions in anesthetized, adrenalectomized dogs. The fact that many investigators have not reported the whealing is probably due to this effect of anesthesia and to the wheals' being less obvious when dye is not present in the plasma.

The demonstration that in the dog autologous plasma is an ideal plasma volume expander and that nonautologous plasma is a relatively inefficient expander has a number of implications. 1. It permits control of an unexpected variable in the design of experiments in which blood volume is to be artificially increased. 2. The finding that autologous plasma is completely retained within the circulation for at least an hour shows that the correction of hypervolemia is surprisingly slow. 3. Some experiments on the circulation, in which transfusions of nonautologous blood,
or reservoirs stocked with nonautologous blood, have been used may be open to re-evaluation in the light of these observations. For example, the rapid loss of plasma from the circulation which has been reported to occur in dogs after large transfusions is presumably due at least in part to the nonautologous "foreign" plasma effect, rather than solely to a homeostatic adjustment.

An unexpected observation was an apparent increase in total plasma protein after autologous transfusion 26 per cent greater than could be accounted for by the amount of protein administered (table 2). This increase, although statistically significant ($p < 0.05$), is sufficiently small to be the result of a systematic error in experimental method. If it is real, however, it may be due either to a release of previously sequestered plasma or to the entry of protein-rich lymph into the circulation.

Preliminary experiments have shown that a similar plasma incompatibility can be demonstrated in the human skin and a study on plasma transfusion in man is in progress.

SUMMARY

The transfusion of 20 ml./Kg. of nonautologous plasma almost always produces in the unanesthetized dog a characteristic reaction, the most prominent features of which are skin whealing, particularly in the neck and groin areas, and the rapid loss of the equivalent of much of the added plasma volume and plasma protein. The whealing is much more evident when the plasma proteins are dyed, e.g., with T-1824. In the more severe reactions, facial edema and generalized whealing are seen, together with lethargy and vomiting. Autologous plasma, on the other hand, does not give rise to the characteristic reaction.

The nonautologous plasma reaction is apparently mediated through the release of histamine. The skin manifestations can be prevented completely, and the systemic responses partially, by pretreatment with mepyramine. A sharp increase in gastric acid secretion often follows the transfusions. The reactions do not appear to be related to the presence of natural erythrocyte isoagglutinins in the plasma.

The results of skin testing show that animals with marked sensitivity to intradermal injections of donor plasma also tend to have severe reactions to transfusions of that plasma.

The experiments lead to the general conclusion that nonautologous plasma is far from being an ideal plasma volume expander, and that the rapid plasma and protein loss occurring after transfusions of such plasma are not entirely due to a homeostatic readjustment in blood volume but also to the occurrence of a reaction to "foreign" plasma.

ACKNOWLEDGMENT

We are indebted to Professor F. C. MacIntosh of this department for his advice and encouragement.

SUMMARIO IN INTERLINGUA

In canes non-anesthesiati, le transfusio de plasma non-autologe in quantitates de 20 ml per kg de peso corpore produce quasi semper un reaction caracteristico. Le plus prominent aspectos de illo es vibices del pelle, specialmente in le areas del collo e del inguine e le perdita rapide del equivalente de un grande parte del plasma addite, tanto in volumine como etiam in contento de proteina. Le vibices deveni multo plus evidente quando le proteinas del plasma es tincturate, per exemplo con T-1824. In le plus sever reactiones, edema facial e vibices de distribution general occurre insimul con lethargia e vomito. Plasma autologe, del altere latere, non resulta in un reaction caracteristico.
PLASMA INCOMPATIBILITY

Le reaction a plasma non-autologe es apparentemente mediate via le liberation de histamina. Le manifestationes cutanee pote esser prevenite completemente e le responsas systemic partialmente per un pretraetamento con mepyramina. Un acute augmento del secretion de acido gastric seque le transfusion in multe casos. Le reactiones non pare esser relationate al presentia de natural isoagglutinationes erythrocytic in le plasma.

Le resultatos de tests cutanee monstra que animales con marcate grados de sensibilitate a injectiones intradermal de plasma ab un donator etiam tende a exhibir sever reactiones al transfusion de ille mesme plasma.

Le experimentos supporta le conclusion que plasma non-autologe es non del toto un expanditor ideal del volumine de plasma e que le rapide perdita de plasma e de proteina plasmatic que occurre post transfusiones de tal plasmas non es exclusivemente le effecto de un re-adjustation homeostatic in le volumine de sanguine sed etiam del occurrentia de un reaction al introduction de plasma "alien."

REFERENCES

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