Effects of the spleen on canine factor VIII levels

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Factor VIII levels were assayed before, during, and after either intestinal resection, splenectomy only, splenic autotransplantation, or splenic homotransplantation in 16 normal and 8 hemophilic dogs. Factor VIII levels in normal dogs subjected either to intestinal resection or to autotransplantation of the spleen fell about 50 per cent during surgery, rose to twice baseline values in the postoperative period, and returned to normal in 4 to 5 days. Factor VIII likewise dropped to about 50 per cent during splenectomy in normal dogs and returned to, but did not exceed, baseline levels by the sixth day. Splenectomy of 3 hemophilic animals had no significant effect on factor VIII levels. Homotransplantation of the spleen from a hemophili to a normal dog did not produce a postoperative rise in factor VIII. Homotransplantation of spleens from normal to hemophilic dogs without cryoprecipitate therapy was difficult to accomplish due to bleeding. In one such instance, factor VIII was detected at 16 per cent of normal within 24 hours of homotransplantation but then fell, and the dog died. However, no postoperative rise of factor VIII was observed in 2 other homotransplantation experiments which resulted in long-term survival of hemophilic recipients. These data suggest to us that the function of the normal spleen in factor VIII homeostasis is one of storage rather than synthesis and indicate that homotransplantation of the spleen is not an effective therapy for hemophilia.

On the basis of both ex corpore perfusion studies and of homotransplantation experience, the spleen, kidney, and liver have been proposed as sites of synthesis or storage of factor VIII. The perfusion of normal spleens with...
hemophilic blood has been shown to increase factor VIII activity in the effluent blood, but due to their limited duration, such experiments did not distinguish between storage or synthesis of factor VIII by the spleen. Results from a total of 5 experiments involving the homotransplantation of spleens from normal dogs to 2 different breeds of hemophilic dogs (Irish setter and beagle) have been interpreted as partially correcting the deficiency of factor VIII. While not entirely proved, synthesis within the spleen has been invoked as the explanation for the sustained increments of factor VIII levels following such a transplantation. The intent of this communication is to report results of experiments attempting to define the role of the canine spleen in factor VIII homeostasis and evaluate splenic homotransplantation as a therapeutic mode in hemophilia.

**Methods**

Eight normal mongrels as well as 8 normal and 8 hemophilic male beagles from the Oklahoma Colony of Canine Hemophilia were used in these experiments. Blood from an additional 17 normal mongrels was used to establish normal values in the factor VIII assay. Prior to any surgical procedure, a prothrombin time, a partial thromboplastin time, and a fibrinogen level were determined by conventional laboratory methods on plasma from each dog. These base-line clotting studies were within normal limits for the mongrels and normal beagles while the partial thromboplastin times reflected the virtually absent factor VIII activity in the hemophilic beagles. All blood samples were collected into plastic syringes by the 2 syringe technique. Hematocrits, white blood cell counts with a peripheral blood smear, and platelet counts were performed by conventional techniques through the experimental period for as long as the dogs survived. Factor VIII activity was measured by the 1 stage kaolin-activated partial thromboplastin time with the use of canine factor VIII-deficient substrate. This method was chosen to avoid the problems encountered when plasma samples from different species are mixed. To illustrate this problem, when normal human test plasma was used with the canine factor VIII-deficient substrate, the activity was less than 10 per cent of the canine control. On the other hand, normal canine plasma with a human factor VIII-deficient substrate demonstrated 5 to 6 times the human activity. These comparative assays suggest different mean normal activities in the 2 species. Fig. 1 is a graph of the means from factor VIII assays on the 8 hemophilic beagles contrasted with the means of assays performed on 17 healthy mongrels randomly selected at the time the beagles were assayed. All hemophilic dogs had less than one per cent factor VIII activity, i.e., not detectable, with the use of the mean base-line factor VIII of the normal animals as 100 per cent activity. Serial determinations of factor VIII activity on these hemophilic dogs from the age of 6 weeks consistently showed similar results. The range of factor VIII in the mongrel group read against the same control was from 45 to 131 per cent. Repetitive assays on the same animal on 7 successive days had a similar variation. In some of the surgical experiments on hemophilic dogs, cryoprecipitate prepared according to the method of Pool and Shannon was used to correct the factor VIII deficiency. In the splenectomy experiments, radiisotope scans of the spleen were performed with the use of an intravenous dose of 3 mc. of technetium heptasulfide colloid. All animals were kept in quarantine for 10 days prior to surgical procedures to insure that they had no underlying disease and remained in good health. Before each operative procedure, the dogs were anesthetized with a methoxyflurane-oxygen mixture administered via a semiclosed system.

**Intestinal resection in normal mongrel dogs.** Resection of 16 cm. of small intestine was performed in 3 normal mongrels. The purpose of this experiment was to establish the effects on factor VIII levels of operative procedures which resembled splenectomy in duration and number of vessels cut and ligated. Factor VIII assays were monitored for 3 days prior to, during, and for 6 days after surgery.

**Splenectomy in hemophilic and normal beagles.** A simple splenectomy with ligation of the splenic hilar vessels 8 cm. from the spleen was performed in 3 hemophilic and 2 normal beagles. Factor surgery. The her thereafter. No e and all later set animals for trans Splenic out subjected to spic procedure of Msolution and per prior to, during, spleens were per formed by retrog Splenic hom plantation was r One normal mon which served as One of the sple
activity in the effluent from normal dogs or beagle) have been reported results from a total of spleens from normal dogs and beagles). While not invoked as the explanation for the abnormality in the transplanted spleens, the activity of factor VIII homeostasis in beagles with hemophilia. Beagles. Factor VIII assays were monitored for 3 days prior to, during, and for 5 days after surgery. The hemophilic dogs were given cryoprecipitate the day of surgery and for 48 hours thereafter. No excessive hemorrhage was noted in any of the hemophilic dogs after 2 days, and all later served as recipients of normal spleens. The 2 normal beagles served as donor animals for transplantation in the hemophiliacs.

Spleenic autotransplantation in normal mongrel dogs. Four normal mongrel dogs were subjected to spleenectomy with reanastomosis of the spleen in the iliac fossa according to the procedure of Marchioro and co-workers. The spleens were immersed in cold lactated Ringer’s solution and perfused prior to transplantation. Factor VIII assays were monitored for 3 days prior to, during, and for up to 10 days after surgery. Radiolabelled spleens were performed in all dogs, and the patency of the vascular anastomosis was confirmed by retrograde arteriograms.

Spleenic homotransplants in normal mongrel and hemophilic beagles. Spleenic homotransplantation was also performed according to the procedure of Marchioro and co-workers. One normal mongrel and 8 male hemophiliacs received homotransplants. The normal dogs which served as spleen donors for the hemophilic beagles were all from the same family. One of the spleenectomized hemophilic dogs served as a donor for a normal spleenectomized
mongrel. Factor VIII levels were monitored 3 days prior to surgery, during surgery, and in the postoperative period for as long as the dog survived. Radioisotope scans of the spleen were performed 2 days after splenic transplantation and at weekly intervals thereafter for as long as the dog survived or until the scan ceased to show radioactivity. Dogs receiving homotransplants were given azathioprine in a dose of 5 mg. per kilogram of body weight beginning 5 days preoperatively with methyl-prednisolone, 5 mg. per kilogram, added on the day of surgery. Both medications were continued into the postoperative period. The dosages of these 2 drugs were adjusted postoperatively in an attempt to maintain the induced lymphopenia. To test the effects of azathioprine administration on factor VIII levels, a normal and a hemophilic beagle were placed on this drug (5 mg. per kilogram) for one month, and daily factor VIII assays, hematocrits, leukocyte counts, and differential smears were made.

Results

Intestinal resection in normal mongrel dogs. Fig. 2 demonstrates the effect of partial resection of small intestine on factor VIII levels in 3 normal mongrels. Factor VIII levels tended to fall during surgery, then rapidly returned to normal and became elevated up to 2.6-fold before they returned to preoperative levels in 3 to 6 days. The dogs had uneventful recovery periods.

Splenectomy in hemophilic beagles. Three hemophilic beagles underwent splenectomy. During the first 2 postoperative days, hemorrhage was controlled by the administration of cryoprecipitate which maintained circulating factor VIII activity at approximately 10 per cent of normal. After the dogs ceased to receive

\[
\begin{align*}
\text{FACTOR VIII - % ACTIVITY} & \\
\text{TIME IN DAYS} & \\
\text{Surgery} & \\
\text{Pre} & \\
\text{Post} & \\
\end{align*}
\]

\[\begin{align*}
\text{Fig. 2. A consistent transient elevation of factor VIII was noted in 3 dogs within 2 days of partial intestinal resection.}
\end{align*}\]
Spleen effects on canine Factor VIII levels

During surgery, and in 3 normal mongrels, factor VIII activity returned to less than one per cent and has remained at that level.

**Splenectomy in normal beagles.** Fig. 3 shows that factor VIII activity dropped significantly with splenectomy in each of 2 normal beagles and then gradually returned to base line in approximately 4 days. No immediate postoperative elevation was observed in either of the 2 dogs. The 5 dogs which underwent splenectomy developed Howell-Jolly bodies and elevated platelet counts.

**Spleenic autotransplantation in normal mongrel dogs.** Fig. 4 is a graph of the mean factor VIII levels at identical intervals before, during, and after splenic autotransplantation procedures in 4 normal mongrel dogs. It is to be emphasized that each of the 4 dogs showed the same pattern. Again it was noted that factor VIII activity fell during surgery. As with the small intestinal resection in the presence of an intact spleen, there was a marked elevation of factor VIII on the first postoperative day which persisted for about 5 days before base-line values were reached. Adequate splenic perfusion was documented in each experiment by isotopic spleen scans, and these results were confirmed by arteriography.

**Spleenic homotransplantation from a hemophilic to a normal dog.** Fig. 5 demonstrates that, as in splenectomy, factor VIII levels fell during the homotransplantation of the spleen from a hemophilic dog to a normal splenectomized mongrel. Normal levels were approached about 4 days following surgery, but no
Fig. 4. Mean factor VIII levels in 4 normal dogs show that autotransplantation of the denervated spleen was followed by a postoperative rise in factor VIII similar to that observed with intestinal resection.

Fig. 5. Homotransplantation of the spleen from a hemophiliac beagle to a normal mongrel was not accompanied by a postoperative rise in factor VIII.
Fig. 6. A radioisotope scan demonstrates adequate perfusion of the homotransplanted spleen from the hemophilic donor beagle to a normal dog.

elevation of factor VIII activity above base-line levels occurred postoperatively. Fig. 6 is a radioisotopic scan which demonstrates adequate perfusion of the homotransplanted spleen 7 days after the operation. This animal received immunosuppressive drugs and did well until the twentieth postoperative day when it became febrile and died 2 days later of sepsis.

*Splenectomy homotransplantation from normal to hemophilic beagles.* The results of these homotransplantation experiments are summarized in Table I. Hemophilic beagles 1 to 5 underwent splenectomy and simultaneously received a normal canine spleen without the benefit of preoperative cryoprecipitate. Intra-abdominal hemorrhaging became evident in hemophilic recipients 1 to 4 in the immediate posttransplant period, and large doses of both cryoprecipitate and type-specific fresh whole blood were given. Because of the cryoprecipitate therapy, the significance of detectable factor VIII activity in postoperative assays from the experiments could not be evaluated and hence are omitted from the table. The fifth recipient was not given pre- or postoperative cryoprecipitate, and 24 hours following splenic homotransplantation, it was found to have a factor VIII of 16 per cent. The level fell as the dog developed shock and died. Recipients 1 to 5 all died of hemorrhage within 4 days of their surgery. Intra-abdominal bleeding was demonstrated at necropsy in each. The first hemophilic recipient's death could be attributed to hemorrhage from the venous anastomosis. In recipients 2 to 5, capillary bleeding appeared to have caused death since
Table I. Results from homotransplantation of spleens from normal to hemophilic beagles

<table>
<thead>
<tr>
<th>Hemophilic recipient</th>
<th>Cryoprecipitate therapy</th>
<th>Preoperative</th>
<th>Postoperative</th>
<th>Pretransplant (%)</th>
<th>Posttransplant without cryoprecipitate therapy</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>No</td>
<td>Yes</td>
<td></td>
<td>&lt;1</td>
<td>18 hr.</td>
<td>Hemorrhaged from venous anastomosis and died on fourth day despite therapy.</td>
</tr>
<tr>
<td>2</td>
<td>No</td>
<td>Yes</td>
<td></td>
<td>&lt;1</td>
<td>18 hr.</td>
<td>Died 4 hr. after surgery from hemorrhage but no major bleeding sites found.</td>
</tr>
<tr>
<td>3</td>
<td>No</td>
<td>Yes</td>
<td></td>
<td>&lt;1</td>
<td>18 hr.</td>
<td>Died 1 hr. after transplant from hemorrhage.</td>
</tr>
<tr>
<td>4</td>
<td>No</td>
<td>Yes</td>
<td></td>
<td>&lt;1</td>
<td>18 hr.</td>
<td>Died 12 hr. after transplant with intra-abdominal hemorrhage.</td>
</tr>
<tr>
<td>5</td>
<td>No</td>
<td>No</td>
<td></td>
<td>&lt;1</td>
<td>18 hr.</td>
<td>Died 36 hr. after surgery from hemorrhage.</td>
</tr>
<tr>
<td>6</td>
<td>Yes</td>
<td>—</td>
<td></td>
<td>&lt;1</td>
<td>—</td>
<td>Died due to cardiac arrest during surgery.</td>
</tr>
<tr>
<td>7</td>
<td>Yes</td>
<td>Yes</td>
<td></td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>Surviving 8 months posttransplantation; immunosuppressives discontinued. Spleen scan continues normal.</td>
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<td>8</td>
<td>Yes</td>
<td>Yes</td>
<td></td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>Surviving 7 months posttransplantation; immunosuppressives discontinued. Spleen scan continued normal for 6 months.</td>
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major bleeding sites were not identified, and the arterial and venous anastomoses appeared intact and patent.

Three additional adult hemophilic beagles were each given the cryoprecipitate derived from 250 ml. of normal canine plasma and then received spleens from normal beagles without concurrent splenectomy. One of these dogs died from cardiac arrest during surgery. The other 2 dogs did well during surgery,
from normal to hemophilic

<table>
<thead>
<tr>
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<tbody>
<tr>
<td>Hemorrhage from venous anastomosis and died on fourth day despite therapy.</td>
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<tr>
<td>Died 4 hr. after surgery from hemorrhage but no major bleeding sites found.</td>
</tr>
<tr>
<td>Died 1 hr. after transplant from hemorrhage.</td>
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<tr>
<td>Died 12 hr. after transplant with intra-abdominal hemorrhage.</td>
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<tr>
<td>Died 36 hr. after surgery from hemorrhage.</td>
</tr>
<tr>
<td>Died due to cardiac arrest during surgery.</td>
</tr>
<tr>
<td>Surviving 8 months posttransplantation; immunosuppressives discontinued. Spleen scan continued normal.</td>
</tr>
<tr>
<td>Surviving 7 months posttransplantation; immunosuppressives discontinued. Spleen scan continued normal for 6 months.</td>
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and cryoprecipitate therapy was continued for 36 hours postoperatively. Twelve hours after the last dose of cryoprecipitate was given, plasma factor VIII activity was no longer detectable in either dog. Daily factor VIII assays in these 2 dogs were consistently less than one per cent during the next 2 weeks. At that time each dog received cryoprecipitate and was subjected to removal of his "native" spleen to test the hypothesis that the hemophilic spleen destroyed or somehow inhibited the activity of factor VIII released from the transplanted normal spleen. During splenectomy, each of the previously transplanted spleens was judged normal in appearance and consistency by direct visualization and palpation. No areas of infarction were seen, and obvious arterial pulsations were evident in the pedicles. Following this second surgical procedure, daily assays of plasma factor VIII continued to be less than one per cent. Factor VIII inhibitor activity was not detected in either dog throughout the entire experiment.

Because it has been recently suggested that the lymphocyte might be a site of factor VIII synthesis\(^\text{17}\) and the 2 long-surviving beagles had an absolute lymphopenia as a result of the immunosuppressive drugs, azathioprine and methyl-prednisolone were discontinued 5 weeks following the splenectomy. The lymphocyte counts soon returned to normal, but despite this, the factor VIII activity has remained less than one per cent. We had earlier demonstrated that prolonged administration of azathioprine (5 mg. per kilogram) to both a normal and a hemophilic beagle for 30 days induced lymphopenia but had no demonstrable effect on factor VIII levels. Weekly radioisotopic scans of the transplanted spleens in the 2 long-surviving hemophilic dogs continued to demonstrate normal uptake. Fig. 7, A shows an isotopic scan of recipient 7 which shows, in...
addition to the liver, a transplanted normal spleen with the dog's own spleen present. Fig. 7, B shows a scan of the same animal 4 months after removal of his native spleen which indicates adequate perfusion of the homotransplant. Currently, 8 months post transplant, this same dog although not receiving immunosuppressive drugs appears healthy and, despite additional proof of adequate splenic perfusion, has no demonstrable factor VIII activity. Recipient 8, also a male hemophilic dog, has survived for 7 months with no rise in factor VIII. His homotransplanted spleen continued to show adequate perfusion for 6 months although he was not receiving immunosuppressive therapy.

Discussion

Intestinal resection was used to assess the effect of surgery not involving the spleen on plasma factor VIII levels. Amundsen and associates observed a postsurgical factor VIII rise in humans similar to our observations in dogs. They suggested that increased circulating catecholamines were the cause. This seems reasonable since it is known that factor VIII increases following exercise, adrenaline injection, and stimulation of hypothalamic areas which activate cells in the adrenal medulla. These observations are strengthened by the report that beta-adrenergic receptor blocking agents prevent the rises after adrenaline injection.

As early as 1939, and then in 1950, it was reported that a decreased bleeding tendency occurred in human hemophiliacs following splenectomy. Gross and co-workers, however, could not demonstrate a lessening of hemorrhagic manifestations after splenectomy in 4 hemophilic patients or 2 hemophilic Irish setters. Our results in hemophilic beagles confirmed their findings. In the normal mongrel, both splenectomy and intestinal resection were associated with a transient fall in factor VIII. We attributed this fall to utilization of factor VIII as the vessels were sectioned and ligated. No rise of factor VIII, however, occurred in the normal dogs following splenectomy. This implied that the spleen is the effector organ for postoperative elevations of factor VIII. The results of Libre and colleagues agree with this proposal since they showed that adrenaline administration to normal humans caused a rise in circulating factor VIII, but adrenaline given to splenectomized individuals did not have this effect.

Although they used a different experimental design, the results of the studies of Weaver, Price, and Langdell resemble our findings. In their cross-circulation experiments, they found that the presence of a normal spleen could acutely raise the factor VIII level in the hemophilic patient. Their experiments, however, did not differentiate between a mechanism of factor VIII storage or synthesis in the normal spleen.

Our splenic autotransplantation data obtained with normal mongrels confirmed the initial fall in factor VIII during splenectomy and the postoperative elevation when the spleen was restored to the circulation. Since the spleen was necessarily denervated during removal from its anatomical position, our experiments suggest a humoral mechanism for the postoperative factor VIII elevation.

The failure of the postoperative rise in factor VIII to occur in the normal splenectomized mongrel which received the spleen from a hemophilic dog resembles the typical factor VIII pattern found in man.

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resembled the results of experiments in which normal dogs underwent splenectomy only. This finding agrees with the hypothesis that the hemophilic patient does not make factor VIII and suggests that the spleen from the hemophilic has no stored factor VIII for release in situations such as the postoperative state.

We were unable to detect persistent plasma factor VIII activity following homotransplantation of normal spleens into 2 hemophilic beagles despite a period of observation extending over several months. The presence of the dog's own spleen did not alter the results as factor VIII did not appear in his blood when his "native" spleen was removed 2 weeks after the transplantation. Also, termination of immunsuppressive drugs several weeks later did not promote the appearance of detectable circulating factor VIII activity.

Hence, although our dogs were offspring of the same family, we cannot confirm the results of Norman, Covelli, and Sise.\textsuperscript{3} Their assay which mixed plasma from 2 species may partially explain this discrepancy. When their values are divided by 6,\textsuperscript{22} the results compare favorably with the factor VIII activity observed by Webster and associates\textsuperscript{26, 24} in similar experiments with the use of an assay system identical to ours in hemophilic Irish setter recipients which survived only a few days after homotransplantation. Marchioro and co-workers\textsuperscript{25} recently reported a splenic homotransplantation experiment in which a hemophilic male beagle from the Oklahoma Colony was the recipient of a normal spleen and developed an insignificant early rise in factor VIII. An additional explanation for the discrepancy is that Norman and colleagues used a group of exclusively female "hemophilic" which could have contained a carrier with very low factor VIII activity. Indeed, the bitch which had the longest surviving splenic transplant had detectable plasma factor VIII prior to surgery. We have observed similar females in our colony and, therefore, chose to use only males in our experiments.

We conclude that the normal spleen stores, rather than synthesizes, factor VIII. Since the single report\textsuperscript{2} of a human transplant was complicated by severe hemorrhage, we would urge that the primary organ site for factor VIII synthesis be identified before the hypothesis of organ homotransplantation as a "cure" for hemophilia is further pursued.

We wish to express our appreciation to Drs. C. G. Gunn, E. Jones, L. Greenfield, and J. Bohr for their support in this project and the staff of the Departments of Surgery and Medicine at the University of Oklahoma Medical Center and the staff of the School of Veterinary Medicine, Oklahoma State University.

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