Blood Coagulation in Acute Iron Intoxication

By Sloan J. Wilson, Helen E. Heath, Paul L. Nelson and G. George Ens

While studying acute iron intoxication in dogs, Reissmann and his associates noticed that just prior to death in dogs no anticoagulant was necessary for collecting blood for various studies. Intestinal bleeding was observed in some experimental animals. Smith, Jones, and Cochran reported that in a fatal case of ferrous iron intoxication the heart’s blood remained unclotted for five hours after death. Scattered hemorrhages were observed.

The observations of these investigators stimulated our interest in the study of blood coagulation in acute iron intoxication. The following factors were observed, using rabbits as the experimental animal: coagulation time, platelets, clot retraction, qualitative and quantitative studies of fibrinogen, fibrinolytic activity, and prothrombin. The defect in blood coagulation was apparently caused by a combination of factors.

Methods

Observations were made on 24 adult rabbits. Usable data were obtained from 16 animals, the remainder dying too suddenly to study completely. All studies were done in the fasting state. Ferrous sulfate in a 10 per cent solution was passed into the stomach through a catheter. The dosage of iron (calculated as iron, not as ferrous sulfate) ranged from 300 to 700 mg. per Kg. body weight. All blood specimens were obtained by cardiac puncture. Determinations were done every 2 hours unless death intervened, blood then being obtained quickly from the aorta or inferior cava.

Serum iron determinations were done by a spectrophotometric adaptation of Barkan’s method. Platelets were determined by the direct citrate method using 3.6 per cent sodium citrate as the diluting fluid. Coagulation times were done by the two-test-tube method, placing 1.0 ml. of blood in each tube and tilting every ½ minute. The retraction of the clot was observed for 24 hours at 37 C. The fibrinogen was determined quantitatively by a spectrophotometric adaptation of the method of Greenberg and Mirolubova. Thrombin was added to assure complete transformation to fibrin. Fibrinolytic activity was determined by the incubation of 1.0 ml. aliquots of plasma at 37 C., the transformation to fibrin being completed by the addition of thrombin. The amount of fibrin remaining in 24 and 48 hours was determined quantitatively. The fibrinolytic activity was also determined qualitatively by observing the clot in the blood incubated for clot retraction. Prothrombin values were determined by the one-stage method of Quick and the two-stage method of Warner, Brinkhous and Smith as modified by Ware and Seegers. The fibrinogen used in the two-stage method was precipitated with cold saturated ammonium sulfate solution. After repeated dissolving in 0.9 per cent NaCl and precipitation with cold saturated ammonium sulfate, the final fibrinogen solution was dialyzed in the cold against 0.9 per cent NaCl, and after centrifugation the pH was adjusted to 7.0.

Results

Serum Iron Levels

Control serum iron levels ranged from 130 to 422 gamma per cent. Increases in the serum iron values were proportional to the dosage of iron.
Figure 1 illustrates the serum iron values in representative experimental animals. These values were greatly increased after the administration of iron, the highest being 42,788 gamma per cent. The serum and plasma were the color of rust.

The survival times of the experimental animals were inversely proportional to the amount of iron placed in the gastrointestinal tract.

Coagulation Time, Clot Retraction, and Platelets

The most striking initial observation was a prolonged coagulation time in all rabbits receiving 400 mg. or more of iron per Kg. body weight. It was noticed (fig. 2) that the coagulation time increased proportionally to the levels of serum iron. The blood did not clot in 48 hours in one animal receiving 600 mg. per Kg. body weight.

The clot retraction was excellent in rabbits receiving 300 mg. of iron per Kg. body weight. Clot retraction was absent (24-hour observation) in all but one rabbit receiving greater amounts of iron.

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SERUM IRON VALUES

![Graph showing serum iron values](image)

**Fig. 1.**—Serum iron levels in rabbits following administration of iron. Iron placed in stomach by catheter at 0 hours.
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Platelet levels were not altered in rabbits receiving 300 and 400 mg. of iron. There was a decrease in all animals receiving larger amounts of iron (fig. 3). Platelet values of less than 100,000 per cu. mm. were observed when iron dosages were 600 mg. or more. The lowest platelet level observed was 12,000.

Prothrombin

Normal prothrombin times (one-stage method of Quick) ranged from 8.5 to 12 seconds. There was a significant elevation in the prothrombin time with

![Diagram of coagulation time after iron administration](image)

**Fig. 2.**—Coagulation times after iron administration. As the amount of iron increased the coagulation times became more prolonged.
Fig. 3.—Platelet values in rabbits after iron administration. A platelet count of less than 100,000 per cu. mm. occurred in all animals receiving 600 or more mg. of iron per kg. body weight.

only one exception. Examples of representative animals are illustrated in figure 4. If the time in seconds was converted to per cent of normal, it will be observed that there was a marked decrease in prothrombin. Prothrombin times longer than 25 seconds are less than 20 per cent of normal. In animals receiving large amounts of iron the endpoint of the prothrombin test was indistinct because of the nature of the fibrin strands. The fibrin would appear as a rust-colored flocculated precipitate.

Because of observations on fibrinogen, the question immediately arose as to whether the increase in the prothrombin time was caused by a defect in prothrombin or fibrinogen. In the one-stage method the fibrinogen naturally occurring in the plasma is relied upon for prothrombin determination. Two groups of tests were done in an attempt to answer this question. One group of tests was a modification of the one-stage (Quick) method, and in another group the two-stage technic was used.

In the first group of tests 2 rabbits were observed (table 1). Oxalated blood was obtained from the heart and the plasma divided into 2 aliquots. Purified thrombin was added to one aliquot to remove fibrinogen, the pro-
thrombin remaining in the plasma. The second aliquot was treated with alumina to remove prothrombin, the fibrinogen remaining. The rabbits were then given 700 mg. of iron per Kg. body weight and in 1½ and 2 hours blood was obtained by heart puncture and oxalated. Alumina and thrombin treated plasma aliquots were then prepared as in the control plasma. Table 1 shows the results of tests performed. The CaCl$_2$ and thromboplastin suspensions were those used in the standard Quick technic for prothrombin; 0.1 ml. of each plasma specimen, CaCl$_2$, and thromboplastin were used in the test. Whenever the postiron “prothrombin” or “fibrinogen” plasma was substituted for similar control preparations, the time of coagulation was prolonged. The postiron fibrinogen, when converted to fibrin, was rust-colored and would flocculate instead of forming strands.

In the second group of tests the prothrombin was determined by both the one-stage and two-stage methods. Coagulation times were also observed. Table 2 gives the pertinent data obtained. Control and postiron observations were made. The postiron plasma was observed 2½ hours after the iron was given. In the two-stage method the final titration dilution was at least 1:100. There was a distinct difference in prothrombin values obtained by the two methods. It is to be remembered that “purified” fibrinogen was used in the
TABLE 1.—Tests of coagulation activity using controls and postiron (PI) alumina and thrombin-treated plasma. Thromboplastin and CaCl₂ were also added. The alumina plasma had no prothrombin with fibrinogen remaining and the thrombin-treated plasma the reverse. There is a defect in both fibrinogen and prothrombin.

<table>
<thead>
<tr>
<th>Control Prothrombin-free plasma plus</th>
<th>Coagulation time (seconds)</th>
<th>Character of fibrin clot</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control Fibrinogen-free plasma</td>
<td>Rabbit 19</td>
<td>14</td>
</tr>
<tr>
<td>Control Fibrinogen-free plasma</td>
<td>Rabbit 20</td>
<td>14</td>
</tr>
<tr>
<td>Control Fibrinogen-free plasma</td>
<td>Rabbit 19</td>
<td>34</td>
</tr>
<tr>
<td>P.I. Prothrombin-free plasma</td>
<td>Rabbit 20</td>
<td>34</td>
</tr>
<tr>
<td>P.I. Fibrinogen-free plasma</td>
<td>Rabbit 19</td>
<td>24</td>
</tr>
<tr>
<td>Control Prothrombin-free plasma</td>
<td>Rabbit 20</td>
<td>22.5</td>
</tr>
<tr>
<td>P.I. Fibrinogen-free plasma</td>
<td>Rabbit 19</td>
<td>79</td>
</tr>
<tr>
<td>P.I. Prothrombin-free plasma</td>
<td>Rabbit 20</td>
<td>62</td>
</tr>
</tbody>
</table>

two-stage method, and not the fibrinogen inherent in the plasma. Again the fibrinogen in the one-stage method would flocculate instead of forming fibrin strands and was rust in color.

Fibrinogen and Fibrinolysis

The quantity of fibrinogen was unchanged in rabbits receiving 300 and 400 mg. of iron per Kg. body weight. Control values ranged between 228 and 410 mg. per cent. With larger amounts of iron the fibrinogen values appeared to be elevated (fig. 5). Difficulty was encountered in interpretation of the elevated fibrinogen values because the fibrin clots were extremely friable, difficult to handle and wash in water to remove excess plasma proteins. The fibrin was a deep rust color and undoubtedly altered the readings in the spectrophotometer.

Because of the rust color of the fibrin, iron determinations were done on fibrin clots before and after iron intoxication. Two rabbits were studied, 700 mg. of iron per Kg. body weight being given. The postiron plasma samples were obtained 3½ hours after iron was given. The fibrinogen in 1.0 ml.

TABLE 2.—Comparison of the prothrombin content of plasma as determined by the one-stage and two-stage methods, control and postiron studies. Prothrombin in percent is "percent of normal."

<table>
<thead>
<tr>
<th>Rabbit</th>
<th>Prothrombin (700 mg. iron per Kg. body wt.)</th>
<th>Control Studies</th>
<th>Postiron Studies</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Prothrombin one-stage</td>
<td>Prothrombin two-stage</td>
<td>I.V. Coag. min.</td>
</tr>
<tr>
<td>No. 21</td>
<td>10.5 sec.</td>
<td>225.5 units</td>
<td>6 min.</td>
</tr>
<tr>
<td></td>
<td>(100%)</td>
<td>(100%)</td>
<td></td>
</tr>
<tr>
<td>No. 23</td>
<td>9 sec.</td>
<td>215 units</td>
<td>6.5 min.</td>
</tr>
<tr>
<td></td>
<td>(100%)</td>
<td>(97%)</td>
<td></td>
</tr>
<tr>
<td>No. 24</td>
<td>10 sec.</td>
<td>170 units</td>
<td>6.5 min.</td>
</tr>
<tr>
<td></td>
<td>(100%)</td>
<td>(76%)</td>
<td></td>
</tr>
</tbody>
</table>
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**Fig. 5.—Fibrinogen studies in rabbits after iron administration.** The elevation is probably false since the fibrin was a deep rust color and altered the spectrophotometric analysis.

amounts of plasma were converted to fibrin with 20 units of thrombin, and the clot thoroughly washed for one hour. In the control plasma the iron content of fibrinogen (measured as fibrin) was 5.0 gamma. The postiron plasma contained 500 gamma of iron in the fibrinogen in 1.0 ml. of plasma. The fibrin clots were a rust color and very friable.

Fibrinolytic studies revealed no lytic action in any animal observed.

**DISCUSSION**

It is immediately recognized that all known factors concerned in blood coagulation were not studied. However, certain definite defects in coagulation were observed in acute iron intoxication. Apparently the faulty coagulation is due to a combination of factors: thrombocytopenia, hypoprothrombinemia, and qualitative changes in fibrinogen.

Thrombocytopenia per se does not produce a prolongation of the coagulation time, but does have an effect on clot retraction. The abnormality of the fibrinogen cannot be ignored as possibly also having an effect on clot retraction, as well as increasing the coagulation time.

The hypoprothrombinemia observed in these studies is not as severe as one might conclude from the results of the one-stage method. This method would indicate a decrease in prothrombin to hemorrhagic levels. The fibrinogen utilized in the one-stage technic is that which is normally contained in the plasma. Thus a defect in the physiologic activity of the fibrinogen with a prolongation of coagulation would be falsely interpreted as a decrease in prothrombin activity. A modification of the one-stage method revealed a defect in fibrinogen as well as prothrombin. The two-stage method for quantitative prothrombin utilizes purified fibrinogen, and the final dilution for
titration decreased the ferric iron coming in contact with the fibrinogen. This method showed that the decrease in prothrombin is not as severe as one might conclude from the one-stage method, and the prothrombin was not depleted to the extent that faulty coagulation could be explained on a basis of hypoprothrombinemia alone.

The physiologic activity of fibrinogen is decreased by the iron content of the plasma. Postiron specimens of fibrin are rust-colored, friable, fragment easily and cause a prolongation of coagulation. Actual iron determination revealed a tremendous amount of iron in the fibrin. The iron and fibrin could not be disassociated by an hour's washing with water. From the observations of Reissmann and his associates, it would appear that the iron detrimental to the physiologic activity of fibrinogen is the non-beta-globulin bound iron which is in the ferric state. This phenomenon probably occurs only after the beta-globulin is saturated with iron.

It is justifiable to conclude, on the basis of our observations, that the principal cause for the coagulation defect in acute intestinal iron intoxication is a chemical change in the fibrinogen, causing a defective fibrin clot as well as a prolongation of the coagulation time. The thrombocytopenia and hypoprothrombinemia play a secondary role.

**Summary**

Acute intestinal iron intoxication was produced in rabbits and the levels of serum were correlated with changes in blood coagulation.

Acute intestinal iron intoxication resulted in a prolongation of the coagulation time or a complete absence of coagulation, thrombocytopenia, hypoprothrombinemia, and qualitative changes in the fibrinogen. Clot retraction was decreased to absent.

The most marked defect occurred in fibrinogen. In the postiron period the fibrin clot was rust-colored, friable, and fragmented easily. The iron content of the fibrin was tremendous. The physiologic activity of the fibrinogen was decreased and coagulation prolonged.

Fibrinolytic studies revealed no increase in the lysis of the fibrin.

The decrease in the physiologic activity of the fibrinogen frequently produced a hemorrhagic level of prothrombin as measured by the one-stage method. The prothrombin as measured by the two-stage method, although decreased, was not in the hemorrhagic zone. Modification of the one-stage method, correlated with the prothrombin values as determined by the two-stage technic, revealed a defect in both prothrombin and fibrinogen.

**Summario in Interlingua**

Acute intoxication intestinal a ferro esseva producite in conilios. Le nivellos del ferro seral esseva correlationate con alterationes in le coagulation del sanguine.

Acute intoxication intestinal a ferro resultava in un prolongation del tem pore de coagulation o in complete absentia de coagulation, in thrombocytopenia, in hypoprothrombinemia, e in alterationes qualitative in le fibrino-
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Le retraction del coagulo esueva reducete usque al puncto de su complete absentia. Le plus marcate defecto occurreva in le fibrinogeno. In le periodo post ferro le coagulo de fibrina esueva de color rubiginose, fricabile, e facile a fragmentar. Le contento de ferro in le fibrina esueva tremende. Le activitate physiologic del fibrinogeno esueva reducete e le coagulation esueva prolongate. Studios fibrinolytic revelava nulle augmento del lyse de fibrina.

Le reduction del activitate physiologic del fibrinogeno produceva frequentemente un nivello hemorrhagic de prothrombina in mesurationes per le methodo uniphasic. Le prothrombina in mesurationes per le methodo biphasic esueva reducete sed non se trovava in le zona hemorrhagic. Un modificacion del methodo uniphasic, correlationate con le valores pro prothrombina determinate per medio del methodo biphasic, revelava un defecto tanto in prothrombina como etiam in fibrinogeno.

REFERENCES

Blood Coagulation in Acute Iron Intoxication

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