Intracorpuscular Red Cell Defect
Masquerading as Valve Induced Hemolysis*

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A case is described wherein hemolytic anemia, hemoglobinemia, and fragmented RBCs coexist with aortic valvular stenosis and insufficiency. The data obtained from "cross RBC survival" procedures indicated that hemolysis resulted primarily from an unidentified intracorpuscular defect despite several clinical and laboratory findings which suggested a cardiogenic mechanism as the etiology. This experience demonstrates the diagnostic usefulness of "cross RBC survival" procedures in cases where valvular disease, fragmented RBCs and intravascular hemolysis coexist, as well as the potential hazard of diagnosing valve-induced hemolysis by exclusion.

The occasional occurrence of chronic intravascular hemolysis following cardiac prosthetic surgery is well documented. In this syndrome the pathophysiologic sequence of events has been postulated to occur as follows: turbulence from valvular deformity fragments erythrocytes and induces intravascular hemolysis. If severe, the resulting anemia increases both cardiac output and turbulence. Long term sequelae, besides anemia, are renal hemosiderosis and iron deficiency through unrelenting urinary iron loss.

Recently, attention has been focused on an apparently identical syndrome occurring in nonsurgical patients with valvular heart disease.

A patient with aortic valvular disease, fragmented erythrocytes, and intravascular hemolysis has been studied. Data indicated an intracorpuscular defect of unknown identity rather than the valvular lesion as the primary etiologic mechanism. This experience is reported to emphasize that a commonly employed approach to differentiating between these mechanisms could eventuate in an unwarranted thoracotomy.

METHODS

Hematologic Studies

Routine hematologic studies were performed using standard methods. The following studies were performed according to published methods: incubation hemolysis, RBC mechanical fragility, RBC life span, glutathione reductase, pyruvate kinase, RBC osmotic fragility, cold agglutination, and Donath-Landsteiner cold hemolysis, acid hemolysis, fetal hemoglobin, RBC sequestration, "sugar water" test, "Heinz body" prep, glucose 6 phosphate dehydrogenase (G-6-PD) activity, and serum vitamin B12.

Red cell fragments (schistocytes) were examined on stained smears and enumerated according to the criteria of Tuffy et al. Hemosiderin in body tissues and fluids was demonstrated by prussian blue staining.

Biochemical and Serologic Studies

The following studies were performed according to published methods: serum complement, haptoglobin, serum iron, iron binding capacity, fecal urobilinogen, and porphobilinogen. Harlico kit No. 64208 was used in testing for urinary heavy metals.

Plasma Hemoglobin

Specimens were obtained twice daily: at dawn prior to patient's arising and at 1 PM after ambulation, stair climbing, and dining. Following atraumatic venipuncture, blood was drawn into a plastic syringe through a No. 19 needle, was placed in a siliconized tube containing a drop of heparin, and plasma was separated immediately by centrifugation. Hemoglobin was determined by the benzidine method.

CASE REPORT

A 52-year-old white man presented in March, 1965, with a history of generalized "swelling" at age eight, a cardiac murmur discovered at age 30, and progressive dyspnea since age 48. Although a heavy smoker, he had had no significant bronchopulmonary disease nor had he restricted dietary sodium nor had he taken cardiac medication. There was no personal or family history of anemia or hematologic disorder and a US Army medical evaluation in 1946 failed to include hematologic data.

For the two years prior to his initial interview he had noticed easy bruisability, intermittent dysuria, and ejaculatory discolored reddish brown. During this interval, his wife had noted intense postcoital vaginal irritation.

Examination of this darkly pigmented man disclosed a
blood pressure (BP) of 140/70 and a regular pulse of 80. The point of maximal impulse (PMI) was 1 cm lateral to the midclavicular line. A systolic crescendo murmur over the entire precordium and a grade III/VI decrescendo diastolic blowing murmur along the left sternal border was heard. He had clubbed fingers and toes since birth as had his grandfather.


During all admissions white blood cell (WBC) counts have ranged from 5,000 to 7,000 mm$^3$ and platelets from 65,000 to 125,000 mm$^3$. Differential has characteristically shown 60 percent lymphocytes and 40 percent polymorphonuclears (PMNs). Hemoglobin and hematocrit have ranged from 11.0 gm percent to 25 to 32 vol percent respectively. Reticulocytes of 3 percent to 8 percent has been consistently noted. Bone marrow aspirates have displayed a marked normoblastic erythrocytic hyperplasia and a mild left shift in granulocyte precursors. Peripheral blood has displayed anisocytosis, poikilocytosis, polychromatophilia, basophilic stippling, crenation, microspherocytosis, and fragmentation (Fig 1). Schistocytes have ranged from 6 to 16/1000 (normal 3/1000). Nucleated red cells have ranged from 1 to 14/100 WBC.

The patient was found to have persistently elevated levels of plasma-free hemoglobin and his urine was found to contain hemosiderin intermittently.

Cardiac catheterization, performed prior to digitalization, disclosed a transaortic valvular gradient of 40 mm Hg and a working diagnosis of hemolytic anemia induced by intracardiac turbulence was made.

Levels of plasma hemoglobin, however, were found to undergo no diurnal variation (Fig 2) and although urine color consistently varied from a translucent yellow at dawn to an opaque reddish-brown by afternoon, no hemoglobin was detected in specimens taken throughout the day. The sediment from afternoon specimens consisted of amorphous brown crystals microscopically indistinguishable from sodium urate. Two milliliters of 0.1 N NaOH was added to an aliquot of afternoon urine; another aliquot was heated over a Bunsen flame. The color of both tubes then approximated that of early morning urine (Fig 3). Porphobilinogen and red cells have been consistently absent from the urine.

Smears of prostatic expressate were stained for iron. These were red-blue by gross inspection and disclosed no red cells microscopically. There was present, however, blue staining material occurring both as widely distributed particles and as feathery deposits throughout the substance of the expressate (Fig 4). Stained smears from two other patients with chronic prostatitis disclosed occasional blue staining particles but no fluffy deposits within the substance of the expressate. Sections of the patient's prostate biopsy failed to take up prussian blue stain.

Serum iron and TIBC values have ranged from 98 to 113 μg percent and 223 to 307 μg percent respectively. Serum vitamin B$_{12}$ was 389 μg/ml. The urine contained no heavy metals. Occult bleeding was not demonstrated. Serum protein electrophoreses, blood cultures and LE preparations were normal. Six percent of sulfobromophthalein (brom-sulphalein) was retained after 45 minutes. Protein-bound iodine (PBI) was 5.8 μg percent. Serum uric acid was 4.0 mg percent. Serum bilirubin has varied from 1.0 to 4.0 mg percent with direct reacting fractions never exceeding 1.2 mg percent. Leukocyte alkaline phosphatase scores were 82 and 210. Fecal urobilinogen was 450 mg/24 hr. Red cell life span was shortened to T 1/2 of 20 days (nor-

Figure 1. Smear of peripheral blood. Note the prominence of fragmented red cells. Crenated forms and microspherocytes are also present. (X 650)

Figure 2. Plasma hemoglobin levels on 11 of 15 days. Levels were determined twice daily on nine of the days. No diurnally varying pattern is demonstrated.

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normal 27 to 38 days) and red cell sequestration occurred with spleen:liver ratio of 1:74.

These data were felt to support the diagnosis of hemolytic anemia and rheumatic heart disease with aortic stenosis and insufficiency.

Additional hematologic studies were performed. Electrophoresis of hemoglobin (Hgb) on cellulose acetate at pH 8.5 revealed only A. alkali-resistant hemoglobin was 0.75 percent of total hemoglobin. Screening tests for G-6-PD were negative. Donath-Landsteiner cold hemolysis was absent. "Sugar water" and acid hemolysis tests (for PNH) were normal on two occasions. Serum complement and control were both 90 percent complement. Hemolysis of red cells began in 0.54 percent saline and was complete in 0.35 percent saline, the normal range. Mechanical fragility studies revealed 11.8 percent total hemolysis. Cells from three control patients underwent 6.51 percent, 6.32 percent and 5.69 percent total hemolysis (normal 8.1 percent to 9.4 percent).

In unsuccessful attempts to expand red cell mass, one week courses of heparin and prednisone were instituted, the latter being terminated after the onset of acute psychosis.

Autohemolysis testing was then performed. The results are recorded in Table 1. Tests for the presence of specific diseases known to cause type 1 autohemolysis were then performed. Glutathione reductase activity was 8.48 moles

**Table 1—Autohemolysis Patterns**

<table>
<thead>
<tr>
<th>Pattern</th>
<th>Whole Blood</th>
<th>Whole Blood + Glucose</th>
<th>Whole Blood + ATP</th>
<th>Enzymatic Deficiency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal hemolysis (%) &amp; &lt;5 &amp; &lt;5 &amp; &lt;5 &amp;</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Type 1 &amp; &gt;5 &amp; Partially corrected &amp; Partially corrected &amp; G-6-PD, GSSG-R, HS, Heinz-body</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Type 2 &amp; &gt;5 &amp; ↑ or no change &amp; Complete reduction &amp; Pyruvate kinase</td>
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<td></td>
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<tr>
<td>Type 3 &amp; &gt;5 &amp; Complete correction &amp; Complete correction &amp; Triose phosphate isomerase</td>
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<tr>
<td>Patient (W.S.) &amp; 16.5 &amp; 11.5 &amp; 9.5 &amp;</td>
<td></td>
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*From Prager, Ref. 15.
GSSG-R is glutathione reductase; HS is hereditary spherocytosis; G-6-PD is glucose 6 phosphate dehydrogenase.
cells in a normal recipient was 11 days. A transfusion of
tagged autologous cells was repeated; T 1/2 was 22 days
(Fig 5).
Studies of RBC membrane sodium and potassium trans-
port are currently in progress.

DISCUSSION

The diagnosis of hemolysis resulting from cardiac
prosthetic surgery ordinarily is not difficult to estab-
lish. The onset of intravascular hemolysis and ery-
throcyte fragmentation usually coincides either with
surgery or with subsequent disruption of cardiovas-
cular architecture.

That deformed cardiac valves themselves can
generate hemolysis was first suggested by the stud-
ies of Brodeur et al.\cite{6,7} which demonstrated short-
ened red cell T 1/2 in some patients with valvular
disease. Also Veneziale\cite{8} has demonstrated hypo-
haptoglobinemia and Eyster\cite{9} has demonstrated
hemosiderinuria and iron deficiency anemia in other
patients with similar lesions. The most convincing
evidence is based on case reports wherein correc-
tive valvular surgery eliminated evidence of hemol-
ysis, RBC fragmentation, and anemia.\cite{10,12} In these
cases, however, the presence of cardiogenic hem-
olysis was not established unequivocally before

initial thoracotomy. None of the investigators em-
ployed "cross RBC survival" techniques. The cases
of Westring\cite{10} and Miller\cite{11} manifested cells with
normal mechanical fragility and in Miller's case, the
autohemolysis test had normal results. Diagnosis
of intravascular hemolysis was based on the pres-
ence of hemosiderinuria in Westring's and Miller's
cases and on the presence of hemoglobinuria in
Ziperovich's\cite{12} case. A cardiogenic mechanism was
suggested in Miller's case by shortened red cell
life span paralleling increased cardiac work from
exercise. In Ziperovich's case, hemolysis began at
commissurotomy, suggesting a similar mechanism.

The great majority of patients with valvular le-
sions do not manifest readily detectable hemolysis.
This form of cardiogenic hemolysis is less dramatic
and is apt to go unnoticed. Even when its presence
is suspected the diagnosis is established with some
difficulty as many diseases generate chronic intra-
vascular hemolysis and fragmented RBCs. Conse-
quently, when these hematologic changes are as-
associated with a valvular lesion, establishing an
unequivocal causal relation is more difficult and
must entail excluding intracorpulsar and other
extracorpulsar processes.

Our patient was shown to have anemia, aortic
stenosis, and aortic insufficiency. Marrow erythro-
cytic hyperplasia, persistent reticulocytosis, in-
creased fecal urobinogen, absent serum haptoglo-
bin, and shortened autologous RBC life span all
confirmed the presence of hemolysis. Elevated lev-
els of plasma hemoglobin suggested intravascular
hemolysis. Erythrocyte fragmentation and other
hematologic characteristics of microangiopathic
hemolysis—red cell crenation and microspherocytosis
accompanying thrombocytopenia—\cite{18} in the absence
of diseases causing microangiopathic hemolytic ane-
mia certainly suggested a cardiogenic mechanism.

Abnormal autohemolysis was demonstrated, G-6-
PD, glutathione reductase, and pyruvate kinase de-
deficiencies were ruled out by appropriate enzyme
assays. Other "Heinz body" anemias were excluded
by acetylphenylhydrazine incubation studies. Other
enzyme deficiencies known to be associated with an
intracorpulsar defect were not assayed, however.

Although urine color varied diurnally, no hemo-
globin was demonstrated in any specimen. The
presence of cardiogenic hemolysis was questioned
after exercise, emotion, and eating—conditions
known to increase cardiac output—failed to alter
the magnitude of hemoglobinemia. Diurnal vari-
ation in plasma hemoglobin levels occurred in Sears
and Crosby's\cite{1} second case despite myocardial insuf-

ficiency.

The "cross RBC survival" studies produced the

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most convincing evidence that an intracorpuscular defect was the etiology of the hemolytic process. The survival of infused chromium-tagged homologous compatible cells was within the normal range while the patient's tagged cells, infused into a normal recipient, manifested a markedly shortened T 1/2. This was interpreted as being inconsistent with a primary extracorpuscular defect, thereby eliminating the likelihood of cardiac surgery correcting the hemolytic process.

Another observation of interest was the existence of stainable iron in prostatic secretions. While the pathogenesis of this phenomenon is unclear, it nevertheless demonstrates a mechanism whereby hemosiderinuria can occur in the absence of detectable hemoglobinuria.

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REFERENCES

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BEETHOVEN THE INFLEXIBLE GIANT

Beethoven (1770–1827) was not even overawed by the greatest poet of his time. In 1812 at Teplitz he met Goethe who somewhat hypocritically complained of everyone's bowing to him. "Never mind" remarked the composer with his usual tact, "after all it is me they are saluting." Once walking arm in arm, they met the Empress with all her court. "Keep as you are" muttered Beethoven, "they must give way to us, not we to them." Deeply shocked, the courtier Goethe broke loose and stood reverentially hat in hand, while Beethoven always punctilious in receiving rather than according respect, rammed his own hat lower down upon his head, and went his surly way. The imperial party courteously divided for him. Then he paused and when Goethe rejoined him said: "Well I've waited for you because I honor and respect you as you deserve, But you did those there too much honor." Then he hauled the old poet over the coals, or, as he phrased it, "thoroughly washed his head."

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