MEDICAL GRAND ROUNDS

PARKLAND MEMORIAL HOSPITAL

4 April 1968

AUTOIMMUNE HEMOLYTIC ANEMIA

Case #1. [redacted] This 55-year old [redacted] woman was seen for routine physical examination by her family internist in [redacted] of 1966. At that time she was completely asymptomatic and the only abnormal finding was a spleen palpable 3 cm beneath the left costal margin, a finding known since 1958 with no evidence of associated hematologic abnormality. Her Hgb at that time was 13.5 gms% and all of her hematologic values were otherwise normal. She was seen approximately 10 days later for a routine pelvic examination and a Hgb done on that day was 10 gms%. This abrupt decrease in red cell values was corroborated and we had the opportunity of evaluating her in our laboratory. When seen she was completely asymptomatic with a negative history. Physical examination revealed a spleen palpable 3 cm beneath the left costal margin. Her Hgb was 12.1 gms%, Hct 35 vols%, WBC 4,700/mm^3 and platelet count 220,000. Reticulocytes were 3.7%. The red cells were normochromic normocytic and marrow revealed normoblastic erythrocytic hyperplasia. Serologic studies revealed a ± Coombs' and a cold agglutinin in the titer of 1:128. All other laboratory data were normal. Within 5 days, without any added therapy, her red cell values had increased to a Hgb of 13.4 gms with a Hct of 41.5 vols% and a reticulocyte count of 1.0. At this time her cold agglutinin titer was 1:4 and her Coombs' test was negative. The initial interpretation was that this represented a possible cold hemagglutinin response although a true acquired autoimmune hemolytic anemia could not be ruled out. She remained asymptomatic until [redacted] of 1966 at which time on routine evaluation she was noted to have slightly more splenomegaly (5 cm) and her Hgb had decreased to 11.5 gms% with a Hct of 34 vols% and a reticulocyte count of 2.1%. Because of the decrease in red cell values she was studied over the next 3 days. Her Coombs' test became progressively more positive and was 4+ (titer 1:256); anti e antibody. During this same interval her spleen increased in size to 13 cm beneath the left costal margin. On the morning of the [redacted] 1966 her Hgb was 9.0 gms% and Hct 29 vols%; by late that evening her spleen had increased in size and her Hgb was 6 gms% with a Hct of 18 vols%. Her cold agglutinin titer remained within normal limits. She was begun on adrenocortical steroids and had a return of her red cell values to a normal level with a decrease in spleen size to approximately 4 cm beneath the costal margin within 2 weeks. Complete evaluation at this time failed to reveal a possible basis for her acquired autoimmune hemolytic anemia. Because of her persistent splenomegaly an elective splenectomy was done in [redacted] of 1966. Exploration of the abdomen at that time failed to demonstrate any evidence of a basis for her disease and histologic interpretation of the spleen failed to add any specific etiology for her acquired hemolytic anemia.

Following her splenectomy her Coombs' test remained positive for approximately 4 weeks and the titer then slowly decreased to the level of + to ± (1:56). The cell values remained stable and the patient was asymptomatic until [redacted] of 1967 at which time she had a mild respiratory infection. On evaluation she was noted to have a Hgb of 11.8 gms% with a Hct of 31 vols% and evidence of a 4+ direct Coombs'. Over the next 4 weeks on no therapy her values slowly decreased to 10.0 gms% and she developed constitutional symptoms. Adrenal steroids were re-started and her red cell values returned to the normal range. Over the next 12 months she was slowly tapered off of her steroids maintaining her red cell values in the range of 15.8 gms%, Hct of 47 vols%.

Her Coombs' has remained 2-3+ during this entire time. Over the past 4 months she has been off adrenal steroids, with no evidence of hemolysis. However, her Coombs' titer is unchanged.

Comments: Interesting features in this patient are: 1.) the evidence of a palpable spleen antedating the evident acute episode by some 8 years suggesting the possibility of previous episodes of autoimmune hemolytic anemia, 2.) the evidence of a transient episode of what was subsequently clear as an acquired autoimmune hemolytic anemia, yet with a disappearance of the positive serologic response, 3.) the non-specific cold agglutinin serologic response, 4.) the occurrence of extremely acute and rapid decreases in red cell values (that can be fulminant and lethal), and 5.) the persistence of the positive Coombs' long after the patient is stable with no longer evident increased red cell destruction.

Masquerading as a Secondary Form of AIHA

Case #2. [redacted] [redacted] Patient is a 65-year old [redacted] female who presented with a 2-month history of weakness, ease of fatigue, dyspnea on exertion and ankle edema. Her history is otherwise negative except that a sister was hospitalized in 1967 for pulmonary tuberculosis.

Physical examination revealed a pale, tachypneic, acute and chronically ill [redacted] woman with pale conjunctivae and mucous membranes. Pertinent positive physical findings included a liver palpable 10 cm beneath the right costal margin but only 15 cm in width. Her spleen was hard and firm, palpable 14 cm beneath the left costal margin.

Laboratory study revealed: Hgb 4.5 gms%, Hct 15.5 vols%, WBC 21,700 with a shift to the left. Platelets were 429,000/mm^3. Smear revealed 4+ anisocytosis, poikilocytosis, and teardrop forms with 24 nucleated RBC's/100 WBC's. Bilirubin was 2.2 mgs% with 0.4 direct. L.A.P. was 216. Serum iron was 135 with IBC of 270. Bone marrow revealed marked erythrocyte hyperplasia with an E:G ratio of 6:1 (normal 1:3 to 1:5). X-rays of long bones revealed increased density. The Coombs' was ++++. Her serum haptoglobin level was normal and her Hgb was normal AA.

The size and consistency of the spleen and profound red cell changes on peripheral smear initially suggested that the diagnosis was agnogenic myeloid metaplasia with an associated acquired immune hemolytic anemia. The patient was begun on prednisone.

Chromium^{51}-labeled erythrocyte studies were carried out and demonstrated a $T\frac{1}{2}$ survival time of 8 days (normal 28-32 days). Sequestration studies demonstrated a spleen:liver ratio of 4:1.

Within 2 weeks of the beginning of steroids her Hgb had risen to 9.3 gms% with a Hct of 25.5 vols% and her spleen was palpable only 8 cm beneath the left costal margin. Over the next 4 weeks her red cell values continued to increase toward normal and at the time she was last seen in the clinic her spleen was no longer palpable, her Hgb 12.5 gms% with a reticulocyte count of 2.4%. She was still on steroids.

Comments: This patient is of interest because: 1.) her massive splenomegaly and profound red cell changes on peripheral smear plus the X-ray report suggested that this AIHA was secondary to an underlying myeloproliferative syndrome of the Agnogenic Myeloid Metaplasia type. 2.) Her sequestration data would support splenectomy responsiveness should steroids fail to maintain her asymptomatic state.

Malignant Lymphoma and AIHA

Case #3. [redacted]. 50-year old [redacted] was seen at the [redacted] in 1961 where a diagnosis of Hodgkin's disease and associated hemolytic anemia was established. Of interest is that he was first evaluated at another hospital in 1957 because of lymphadenopathy and a biopsy was interpreted as sarcoidosis. He was treated with steroids. In 1960 he apparently manifested a hemolytic anemia and an elective splenectomy was carried out in [redacted] of 1960. In 1961 he presented with evident generalized lymphadenopathy and constitutional symptoms and a biopsy demonstrated Hodgkin's. The previously noted hemolytic anemia could not be documented. He was treated with nitrogen mustard and steroids in 1961 and 1962 and again 1963 because of recurrent adenopathy and the development of a Coombs' positive hemolytic anemia. In spite of his steroids and intermittent nitrogen mustard his autoimmune hemolytic anemia became severe and symptomatic, although evidence of his malignant lymphoma was limited to cervical lymphadenopathy. Serial evaluation over several admissions revealed that the neck nodes in the left cervical region recurred in association with each episode of increased red cell destruction; with the disappearance of these nodes following mustard the hemolysis would decrease although his Coombs' remained positive for approximately 1 year at which time it became negative.

On this basis in [redacted] of 1963 he was begun on an extensive course of radiation to the nodes of the left neck. Subsequent to this his cervical adenopathy did not recur and the hemolytic anemia disappeared, in spite of new disease in other areas and a subsequent downhill course and death in 1967.

Impression: The patient is of interest because of: 1.) the not uncommon mis-impression of sarcoidosis initially on the first biopsy. 2.) The relationship of his autoimmune hemolytic anemia to a specific lymphomatous infiltrate with good response to local therapy strongly suggests a site of formation of the erythrocyte coating antibody.

SLE and AIHA

Case #4. [redacted] This 36-year old [redacted] female was first admitted to [redacted] in 1963 at which time she gave a history of epilepsy since age 17 (had had normal EEG previously); malar rash, anorexia and weight loss, and polyarthritis involving hand, wrist, and knee joints about 3 months' duration; she was noted to have malar depigmentation and rheumatoid changes of PIP, MCP joints. Laboratory studies revealed anemia, leukopenia, increased sed rate and hypergammaglobulinemia; latex fixation, SSCA, LE preps, and ANF's were abundantly positive. Prior to institution of therapy she developed high fever, overt psychosis, and seizures; all this was controlled with high dose steroids, phenobarbital and Dilantin. At this time renal function studies and urinary sediment were normal.

Evaluation of her anemia demonstrated increased reticulocytes, increased pigment turnover and positive direct Coombs'. Because of her active SLE she was started on steroids with a good response in her hematologic values. She was followed in arthritis clinic for the next 2 years on varying doses of Prednisolone (20-40 mg) and intermittent salicylates with persisting arthralgias (rarely objective joint findings) and psychotic behavior. Two acute flare-ups of arthritis of hands, elbows, hips, knees, and ankles necessitated hospitalization during 1965. On both occasions symptoms and signs abated with increasing her steroid dosage. During this time she developed severe leg ulcers.

She was admitted to [redacted] in [redacted] 1966 with a 2-week history of chills, fever, cough, and left pleuritic chest pain. A non-cavitating LUL infiltrate and chronic leg ulcers were noted. Initial treatment was methicillin, Kantrex, and Chloromycetin. Sputum studies for AFB were negative (including subsequent culture results). Bronchoscopy was unyielding. During [redacted] and [redacted] 1966, the patient was treated with antituberculous medications on the basis of the X-ray appearance and lack of response to treatment for pyogenic organisms. However, the pulmonary lesion progressed in the face of this treatment and she was transferred to PMH for surgical resection of the LUL in [redacted] 1967. Thoracic Surgery felt she was a poor candidate for surgery so she underwent percutaneous needle biopsy of the lung instead. Material aspirated from the lung yielded North American Blastomycosis on culture. A course of Amphotericin (2.5 gm) was given from J[redacted] to [redacted] 1967 with gratifying response.

She was discharged in [redacted] 1967, but fell on her way to the parking lot and fractured her right hip; this was managed on the Orthopedic Service by Buck's traction and early ambulation. This admission was complicated by a RLL pneumonia which responded to Keflin. She was subsequently admitted with cellulitis and profound azotemia and expired in [redacted] 1967.

Comment: Acquired autoimmune hemolytic anemia commonly complicated systemic lupus erythematosis. As in all of the so-called symptomatic types of AIHA, the treatment of the primary disease is the appropriate focus of attention. In this institution Evan's syndrome (combination of immune red cell destruction and an associated ITP picture) virtually guarantees SLE.

Reticulocytopenia in AIHA

Case #5. [redacted] This 4½-year old [redacted] girl was evaluated on [redacted]/67 because of severe and progressive anemia unresponsive to oral iron. Apparently previous work-up had been negative and the only recorded finding was a Hgb of 7.0 gms%. The child was begun on oral iron and 2 weeks later the Hgb was 4.0 gms%. The complete history was negative.

Examination revealed an extremely pale child, afebrile and in no distress. Positive findings included a soft hemic systolic murmur over the precordium and hepatosplenomegaly, 4.0 and 3.0 cm, respectively. There was no rash, icterus, bruises, or petechiae.

Laboratory:

Hgb 4.0 gms; WBC 3800/mm^3; platelets 398,000/mm^3. The reticulocytes were 0.1%. Peripheral smear revealed marked spherocytosis. The Coombs' was +++ repeatedly. The remainder of the laboratory data was within normal limits including L.E. preps on ANF.

Course:

The child was transfused with packed cells X 2 and prednisone (40 mg per day) instituted. After 6 weeks of therapy the Hgb was 5.0 gms. There was no change in reticulocytes. She was again transfused, and steroid dose was decreased. Within 1 week the direct Coombs' was negative, and there was an increase in reticulocytes and in Hgb concentration. Steroids were progressively decreased and discontinued over a 10-week period without reappearance of autoimmune hemolytic anemia. After approximately 12 weeks off therapy, the patient was rehospitalized with a mild anemia (8 gms). The reticulocyte count was again less than 0.1%, WBC's 1800 with normal differential, platelets 311,000. The direct Coombs' was 1+, the marrow essentially unchanged. All other studies were again normal or non-contributory. The patient was again transfused and prednisone reinstituted.

Hemolysis with Penicillin

Case #6. [redacted] The patient is a 36-year old [redacted] woman with a long history of alcoholism and mental illness, who was admitted [redacted]/67 because of fever, confusion and tremulousness. She had been consuming large quantities of wine daily for several weeks, but had been well until the onset of a sore throat one week before admission. For two days prior to admission she complained of fever and headache, and she vomited several times. On the day of admission she became tremulous, confused, and combative, and apparently had a grand mal seizure.

Her past history revealed many episodes of confusion and tremors following alcoholic sprees, increasingly more frequent since 1958. She repeatedly gave evidence of erratic and irresponsible behavior and was committed to the [redacted] twice in 1958 and 1962.

On admission she had temperature of 104°, pulse 128, and was partially disoriented. Her pharynx was reddened and her neck supple. The liver was palpable 3 fingerbreadths below the right costal margin and was mildly tender.

Lumbar puncture showed 187 WBC, 71% mononuclears, 76 mgs% protein, 70 sugar with 130 blood sugar, non-reactive VDRL, negative smear and culture. WBC was 10,000, Hgb 11.4 gms%, Hct 37, bilirubin 2.2, 0.8 direct, alkaline phosphatase 7.2, TT 8.3, CF 3+, SGOT 660. BUN 5, creatinine 1.2 mgs%, Na 137, K 3.3, Cl 90, CO_2 26.

Within 36 hours of admission her temperature spiked to 105°, disorientation and confusion worsened and marked rigidity developed. She developed vague lateralizing signs, and carotid angiograms showed suggestion of a L anterior cerebral mass lesion. On [redacted]/67 treatment for a suspected brain abscess was begun with 24,000,000 units penicillin and 1 gm Achromycin IV daily. Electroencephalogram and brain scan were negative.

Her condition worsened steadily for the next 9 days, with development of definite hyperreflexia on right, dysconjugate gaze, and unresponsiveness. On [redacted], she had a generalized seizure. Thick tracheal secretion and hypoxemia led to intubation and tracheostomy on [redacted]. Repeat carotid angiograms were unchanged. Repeat LP's showed increasing WBC from 256 to 975, with 60-97% lymphs. CSF protein rose to 205 mgs% and sugars remained normal. Following tracheostomy she gradually improved and became alert. By [redacted] her fever was down to 99° and she was able to sit up and eat.

Early in this stormy course her Hgb dropped sharply with a mounting reticulocytosis. Three transfusions were required to maintain a Hgb of 8-9 gms%.

	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
Hgb	11.4	8	6	7.1	8.3	7.2	8.6	8.8	9.2	10.1
Hct	37		21		27	23		28		34
Retics			2%	8.2%		10%	8%	3.8%	2.6%	4.0%
WBC	10.0		16.8	28.1	13.8	18.8	21.0	25.0	18.3	12.9
Coombs'-direct				Neg.	Neg.	±	+	+	±	Neg.
Coombs' with penicillin						+	++			
G6PD		Normal								Defic.
Bilirubin/dir.		2.6/1.4		3.4/1.8	3.6/1.6		1.0/0.6			

On [redacted], the 19th day of penicillin therapy, the patient developed a positive direct Coombs' which was accentuated by the addition of penicillin to the patient's serum and marked cells. An indirect Coombs' became positive with the addition of penicillin to the incubation of

patient's serum and control RBC's. Penicillin was stopped on [redacted] and Keflin substituted. Tetracycline, phenobarbital, vitamins and Neosporin ophthalmic ointment were continued.

The patient became afebrile and strong by early April, but continued to be disoriented and confused. She was discharged to [redacted] on [redacted] with a diagnosis of acute and chronic brain syndrome.

Alphamethyldopa Hemolysis

Case #7. [redacted] This 67-year old [redacted] was admitted to [redacted] for the first time on [redacted] 1968. In the 6 to 8 weeks prior to admission, she had consulted dermatologists receiving systemic Aristocort therapy without benefit. For the 18 months prior to admission, she had taken Aldomet 250 mg q.i.d. for control of benign essential hypertension. At her last outpatient visit ([redacted], 1967), the patient was normotensive with a normal complete blood count.

Admission physical examination showed only moderate pallor and some excoriations of the skin. Laboratory data on entry as follows:

> Hgb 9.4 gms%, Hct 26%, reticulocyte count 14.6%, WBC (and differential), platelet count, bilirubin, liver function tests, serum iron, and serum protein electrophoresis were normal. Direct Coombs' test 3+.

Aldomet was discontinued and at the time of discharge ([redacted] 1968), the patient was feeling better and studies showed Hgb 10.7 gms%, Hct 30%, reticulocyte count 6%.

By [redacted] 1968: Hgb 13.0 gms%, Hct 46%, reticulocyte count 0.8%, direct Coombs' 3+.

Antiglobulin Positive Test and Pernicious Anemia

Case #8. [redacted] 61-year old [redacted] male was admitted [redacted]/67 with 2-month history of anorexia with an associated 30-pound weight loss and weakness and ease of fatigue; all with an abrupt onset. During this time he also noted numbness and tingling of his feet and "loss of feeling" in the palms and soles of approximately 6 weeks' duration. Remainder of history non-contributory.

Examination reveals a well developed, well nourished man with pale conjunctiva and mucous membranes. The tongue was smooth and manifested atrophic papillae. Neurologic examination revealed loss of vibratory and positional sense in lower extremities and decreased 2 point discrimination.

Hgb 6.2 gms%, Hct 19 vols% with anisocytosis, poikilocytosis and macroovalocytosis. WBC 4150/mm^3 with hypersegmentation. Platelets 163,200/mm^3. Reticulocytes 1%. Total serum proteins 7.8 gms% with albumin 4.5 and globulins 3.3 gms. Remainder of lab studies within normal limits as were complete GI X-rays except for: T.S.I. 287 µg% and TIBC 325. Gastric juice pH 6.75 fasting with rise to 7.0 with maximum histamine stimulation (no free acid).

Serum Vitamin B_{12} 65 μμg/ml. (160-660). Serum folic acid 17.5 nanograms. Schilling less than 1%; 7% after Intrinsic Factor. Coombs' +. Marrow revealed megaloblastic dyspoiesis.

Patient was diagnosed as pernicious anemia and treated with Vitamin B_{12} with reticulocyte peak to 25.2% on [redacted]/67 (day 6 of treatment). Coombs' at that time was ± and became negative thereafter. Current red cell values 14.7 gms%, Hct 46 vols%, Coombs' negative.

Carbohydrate Induced Hyperlipemia and Positive Coombs'

Case #9. [redacted]. 39-year old [redacted] woman seen for evaluation of a normochromic normocytic anemia. Clinical history was unrevealing except for a history of low back pain and a history of hypertension in 8 siblings. Physical examination revealed B. P. of 120/90, lipemic retinal vessels and a liver edge just palpable at the RCM.

Laboratory evaluation revealed Hgb 9.0 gms%, Hct 29 vols%, reticulocytes of 2.2%. The red cells were normochromic and normocytic. Total lipids were 3644; the cholesterol 360. Repeat revealed the triglycerides were 2160 and lipoprotein electrophoresis demonstrated absence of chylomicron bind, a normal α and β band with a marked increase in the pre-beta lipoprotein band. The Coombs' was ++, non-gamma.

IDENTIFICATION OF HEMOLYSIS

Evidence of Erythrocyte Damage:

Spherocytosis
Increased Autohemolysis
Increased Osmotic Fragility
Increased Mechanical Fragility
Erythrophagocytosis

Evidence of Increased Hemolysis

Shortened Red Cell Survival (Cr^{51}, DFP^{32})
Increased Carbon Monoxide Output
Increased Fecal Urobilinogen
Elevated L.D.H.
Decreased Haptoglobin
Increased Urine Urobilinogen
Elevated Serum Iron and Increased Transferrin Saturation
(↑ Plasma Hgb and Methemalbumin)
(Hemoglobinuria)
(Splenomegaly)

Evidence of Repair

Reticulocytosis
"Shift" Reticulocytes
Nucleated RBC's
Marrow Erythrocytic Hyperplasia

Laboratory Studies:

The diagnostic criteria of this type of hemolysis is evidence of a positive antiglobin test:

Coombs' Test:

1. Moreschi, C.: Neue Tatsachen über die Blutkörperchen-agglutination. Zbl. Bakt., Abt. I. Orig. 46:49, 1908.
First demonstrated that erythrocytes sensitized with heterologous sera could be agglutinated by antibodies formed against that heterologous protein.

2. Coombs, R. R. A., Mourant, A. E. and Race, R. R.: A New Test for the Detection of Weak and "Incomplete" Rh Agglutinens. Brit. J. Exp. Path. 26:255, 1945.
Introduced the method for detection of "incomplete" antibodies which are so named because they lack the property of causing direct agglutination in vitro. In essence the test identifies erythrocytes coated with this type of antibody by first washing the cells in saline to eliminate the surrounding plasma or serum and then by demonstrating agglutination when the cells are suspended in an anti-human globulin serum - i.e. Coombs' serum.

3. Bohnen, R. F., Ultmann, J. E., Gorman, J. G., Farhangi, M. and Scudder, J.: The Direct Coombs' Test: Its Clinical Significance. Ann. Int. Med. 68:19, 1968.
A recent review of the clinical circumstances of a positive Coombs' test.

Measurement of Red Cell Destruction:

Red Cell Survival and Sequestration Data:

4. Mollison, P. L.: Further Observations on the Normal Survival Curve of Cr^{51} Labeled Red Cells. Clin. Sci. 21:21, 1961.
Although the techniques for evaluation of red cell survival are considered "standard", this paper reviews the foibles and artifacts induced and sources of error and represents a good reference paper on the entire problem.

5. Korst, D. R., Clatanoff, D. V. and Schilling, R. F.: External Scintillation Counting Over the Liver and Spleen After Transfusion of Labeled Erythrocytes. Clin. Res. Proc. 3:195, 1955.
Demonstrated that spleen to liver ratios in excess of 3:1 represented the degree of splenic sequestration compatible with excellent response to splenectomy.

6. Jandl, J. H., Greenberg, M. S., Yonemato, R. H. and Castle, W. B. Clinical Determination of the Sites of Red Cell Sequestration In Hemolytic Anemias. J.C.I. 35:842, 1956.
Popularized the Index of Sequestration as a means of deciding upon splenectomy:
Index of Sequestration = $R_{50}-R_0$ (where R is the ratio of external counts of spleen (or liver) at day 1 and again when $T\frac{1}{2}$ precordium time reached.

Normal Spleen Index is 30-60
Normal Liver Index is 20-35
For anemic patients a spleen index of less than 30 is "subnormal".
Index: 60-100 mild to moderate splenic sequestration
over 100 marked splenic sequestration

Other formulae have been developed to attempt to predict response to splenectomy. The Sequestration Index of Jandl in Veegers series of 23 cases correctly identified 17 patients who would respond and 2 who would not. In 3 patients prediction was incorrect, one had low spleen index and responded and 2 had high index and failed to respond. Allgood and Chaplin (see below) had similar problems.

7. Veeger, W., Woldring, M. G., Van Rood, J., Eernesse, J., Leeksma, C.H.W., Verloop, M. C. and Neeweg, H. O.: The Value of the Determination of the Site of Red Cell Sequestration In Hemolytic Anemia As a Prediction Test for Splenectomy. Acta Med. Scand. 171:507, 1962.

In recalculating recorded data one can predict a beneficial response to splenectomy where the external counting reveals a.) rising counts over the spleen during the period of T_0 to $T_{\frac{1}{2}}$ and b.) where a S:L ratio of 3:1 is achieved. Probable responses are suggested by S:L ratios over 2.3:1. Lesser degrees of sequestration do not preclude a response since other predictive aspects are undoubtedly of significance.

Red Cell Destruction:

Carbon Monoxide Output:

8. Sjöstrand, T.: Endogenous Formation of Carbon Monoxide In Man Under Normal and Pathological Conditions. Scand. J. Clin. Lab. Invest. 1:201, 1949 and Acta Physiol. Scand. 22:137, 1951.

9. Coburn, R. F., Williams, W. J. and Forster, R. E.: Effect of Erythrocyte Destruction in Carbon Monoxide Production In Man. J.C.I. 43:1098, 1964.

These papers have provided the evidence that CO production in man comes mainly from hemoglobin catabolism and that this can be simply and accurately measured in man. In those hemolytic states where hemoglobin catabolism occurs (extravascular hemolysis primarily), this measure provides the opportunity to evaluate destruction rates from day to day or hour to hour.

For each mole of hemoglobin catabolized the cleavage of the porphyrin ring yields one mole of CO.

Normal value: less than 0.0008 cc of CO/gm Hgb/ml blood

Haptoglobins:

10. Giblett, E. R.: Haptoglobin: A Review. Vox Sang. 6:513, 1961.

11. Reerenk-Brongers, E. E., Prins, H. K. and Krijinen, H. W.: Haptoglobin and Increased Hemolysis. Vox Sang. 7:619, 1962.

Two excellent reviews of the biology and utilization of haptoglobins.

Estimation of Repair:

12. Hillman, R. S. and Finch, C. A.: Erythropoiesis: Normal and Abnormal. Semin. Heme. 4:327, 1967.

An excellent review of the kinetics of reticulocyte release and the means of evaluating rates of erythropoiesis.

a.) Reticulocyte Index = % Retics X $\frac{\text{Patient's Hct}}{45}$
(to correct for level of Hct)

The Normal Index is 1.

b.) Correction for Reticulocyte Shift:
Correct for the lengthening of blood reticulocyte maturation time:

Hct level (vol%)	Circulating Maturation Time (days)
40 - 45	1.0
35	1.5
25	2.0
15	2.5

c.) Level of Erythrocytic Hyperplasia of Marrow
Normal E:G ratio is 1:3 to 1:5

SWMS - Acquired Autoimmune Hemolytic Anemia - 1963 - 1967

- Warm Type Antibody

	Males	Females	
Idiopathic -	2	8	
Secondary -			
CLL -	6	2	
Malignant Lymphoma -	5	4	
Ca Ovary -		1	
SLE -		4	
Drug Associated -	2	2	
	15	21	36

Clinical Features:

13. Dameshek, William and Schwartz, S. O.: Acute Hemolytic Anemia (Acquired Hemolytic Icterus, Acute Type). Medicine 19:231, 1940.

The first description in the U. S. and a major stimulus to the study of this type of hemolysis throughout the world.
The definitive study however is:

14. Dacie, J. V. The Hemolytic Anemias II The Autoimmune Hemolytic Anemias. Grune and Stratton, pp. 341-707, 1962.

This entire volume serves as the best general reference and includes Dacie's experience with 129 cases studied during years 1947 and 1961.

15. Allgood, J. W. and Chaplin, H.: Idiopathic Acquired Autoimmune Hemolytic Anemia. Amer. J. Med. 43:254, 1967.

A current but not very revealing survey of 47 cases (years 1955 through 1965) seen at Washington University. The major contribution is the data demonstrating that the absence of selective splenic sequestration in 4 of 7 patients did not preclude a good response to splenectomy.

16. Osgood, E. E.: Antiglobulin-Positive Hemolytic Anemias. Arch. Int. Med. 107: 313, 1961.
Report of 104 cases personally followed by Dr. Osgood.

17. Dacie, J. V.: Prognosis In Autoimmune Anemia. Aust. Ann. Med. 12:11, 1963.
Of 50 patients followed over 1 year with AIHA: 23 died of their disease; 10 underwent clinical and hematologic cure; the remainder had ongoing active disease.

AGGLUTINATION - 37°C
ANTI ERYTHROCYTE ANTIBODIES

Method	Detects	Cause
Saline Agglutination Method	Bivalent or Complete Antibodies 19S - IgM	- Agglutination in vivo - Hepatic Sequestration
High Protein (30% Bovine Albumin) Method	Detects most bivalent and univalent or incomplete antibodies. (Fails to detect Kell, Duffy and Kidd Factors)	Conglutination
Coombs' Antiglobulin Method	Univalent or Incomplete Antibodies 7S - IgG (Fails to detect water soluble Ab as Lewis)	- Agglutination of cells coated with globulin - Splenic sequestration
Enzyme Method (papain) (Others: Trypsin, Ficin, Bromelin, etc.)	- Enhances detection of many univalent antibodies (Rh, Lewis, P systems) - Destroys M and N antigenic sites and Duffy	

Antibody Studies:

Specificity:

18. Race, R. R. and Sanger, R.: Blood Groups In Man. F. A. Davis Co., Phil. 1962.
The best review of blood group data.

The generally accepted concept was that the antibody in cases of AIHA was non-specific, in essence directed against some hypothetical antigen possessed by all human red cells indifferently since antibody could be eluted from the cell surface and yield agglutination of donor cells. The initial clarification came from the studies of Weiner:

19. Weiner, W., Battey, D. A., Cleghorn, T. E., Marson, F.G.W., and Meynell, M. J.: Serologic Findings In A Case of Hemolytic Anemia, With Some General Observations On the Pathogenesis of This Syndrome. Brit. Med. J. ii, 125, 1953.

This was the first clear identification of a true specific "warm" antibody. Since this initial observation many specific anti-Rh antibodies have been demonstrated in the sera and cell eluates from patients with AIHA. These have been as a single entity or as mixed antibodies or with non-specific components. The non-specific antibody however is the most common and has represented almost 60% of the cases.

20. Celano, M. J. and Levine, P.: Anti-LW Specificity In Autoimmune Acquired Hemolytic Anemia. Transfusion 7:265, 1967.

They recently demonstrated anti-LW in 6 unselected cases of AIHA. The LW antigen exists randomly in both Rh positive and negative cells and is very difficult to test for. The serologic data strongly suggests that the presence of anti-LW, an antibody that is considered by Weiner to be directed against the "nucleus of the Rh-Hr substance", will explain most if not all the heretofore "non-specific" data.

Quantitation:

21. Evans, R. S. and Duane, R. T.: Acquired Hemolytic Anemia. I. Relation of Erythrocyte Antibody Production to Activity of the Disease. Blood 4:1196, 1949.

22. Evans, R. S.: Autoantibodies In Hematologic Disorders. Stanford Med. Bull. 13: 152, 1955.

Evans has been the most vocal proponent of the use of a given antiglobulin titer to follow the course of the hemolysis and he has used it to predict responsiveness to therapy. (In addition these papers delineate the occurrence of associated thrombocytopenia which has come to bear the eponymic designation of "Evans' Syndrome".)

Clinically quantitation has not been fruitful as a guide to severity or responsiveness of AIHA. A good review of the problem, especially as it relates to the multitude of available Coombs' sera and their lack of standardization was written by Dr. Sol Haberman.

23. Haberman, S.: On the Specificity and Reactivity of Coombs' Antiglobulin Sera. Blood 13:688, 1958.

Mechanisms of Antiglobulin Positive States:

A. Non-Specific Non-Immune Circumstances:

24. Sutherland, D. S. and Eisentraut, A.: Direct Coombs' Test In Lead Poisoning. Blood 11:1024, 1956.

The positive Coombs' was noted in the reticulocyte-rich layer and is now known to occur simply with reticulocytosis. Reticulocytes are transferrin coated and most Coombs' sera available are simply anti-globulin preparations so that interaction under this circumstance is not unusual. In addition, the transferrin coated reticulocyte is known to have surface metalo-ion present:

25. Jandl, J. H. and Simmons, R. L.: Agglutination and Sensitization of Red Cells By Metallic Cations: Interactions Between Multivalent Metals and Red Cell Membranes. Brit. J. Heme. 3:19, 1957.

B. Isoimmunization Induced:

Since these are well known and classic potential events following transfusion only two features should be stressed:

1. Delayed Reactions May Occur:

This has been best recorded with the Kidd Antibody which is an added problem because of the technical difficulties in identification of Kidd in routine blood banking.

26. Rauner, T. A. and Tanaka, K. R.: Hemolytic Transfusion Reactions Associated With the Kidd Antibody (Jk^a). NEJM 276:1486, 1967.

Evidence of the Kidd sensitization may not be seen for several days or even a few weeks, when increased destruction then is identified.

27. Joseph, J. I., Awer, E., Laulecht, M. and Scudder, J.: Delayed Hemolytic Transfusion Reactions Due to Appearance of Multiple Antibodies Following Transfusion of Apparently Compatible Blood. Transfusion 4:367, 1964.

2. Isoimmunization may occur in the absence of demonstrable incompatibility in the pre-transfusion screening:

28. Fudenberg, H. and Allen, F. H., Jr.: Transfusion Reactions In the Absence of Demonstrable Incompatibility. NEJM 256:1180, 1957.

29. Walker, P. C., Jennings, E. R. and Monroe, C.: Hemolytic Transfusion Reaction After Administration of Apparently Compatible Blood. Amer. J. Clin. Path. 44:193, 1965.

The basis for the failure to identify the event of isoimmunization appears due to:

a.) Antibodies may be fixed in the tissues, especially the spleen.
b.) There may be a very low initial titer which is not detectable but which is followed by a prompt anamnestic reaction. This can lead to a delay of 7-14 days in the apparent hemolysis.
c.) The antibody titer may be too low to detect but it may still be a potent physiologic sensitizer of the erythrocyte membrane.

30. Croucher, E. E., Crookston, M. C. and Crookston, J. H.: Delayed Hemolytic Transfusion Reactions Simulating Autoimmune Hemolytic Anemia. Vox Sang. 12:32, 1967.

C. Drug Induced:

Several mechanisms mediated by drugs exist:

1. Presence of Antigen-Antibody Complexes On Red Cell:

31. Ley, A. B., Harris, J. P., Brinkley, M. and Liles, B.: Circulating Antibody Directed Against Penicillin. Sci. 127:1118, 1958.

32. Petz, L. D. and Fudenberg, H. H.: Coombs'-positive Hemolytic Anemia Caused by Penicillin Administration. NEJM 274:171, 1966.

33. Swanson, M. A., Chanmougan, D. and Schwartz, R. S.: Immunohemolytic Anemia Due to Antipenicillin Antibodies. NEJM 274:178, 1966.

Penicillin is the prototype of this reaction. The Coombs' test is positive only when both drug and antibody coexist on the red cell surface. Here penicillin (in high concentrations) acts as a hapten reacting with a protein moiety to yield antipenicillin antibody. An antigen-antibody reaction results and the erythrocyte surface is injured. This is not specific for the red cell membrane and has been best described as an "innocent bystander phenomenon". Red cell injury and destruction does result. Destruction ceases when drug is discontinued. Thus antibody alone in the absence of drug yields no effect. This is 19S Antibody.

34. Levine, B. B.: Immunologic Mechanisms of Penicillin Allergy. NEJM 275:1115, 1966.

A good review.

2. Presence of Complement Dependent or Activated Destruction:

35. Shulman, N. R.: Mechanism of Cell Destruction In Individuals Sensitized to Foreign Antigens and Its Implications In Autoimmunity. Ann. Int. Med. 60:506, 1964.

Stibophen is the prototype of this reaction. The interaction is similar to the above except that complement is a critical component. In this circumstance the Coombs' may remain positive due to persistence of complement on the erythrocyte surface long after the drug-antibody complex can be demonstrated.

3. Presence of Antibody Globulin Coating of Red Cell:

36. Corstairs, K. C., Breckenridge, A., Dallery, C. T. and Worlledge, S. M.: Incidence of a Positive Direct Coombs' Test In Patients on α-Methyldopa. Lancet 1:133, 1966.

37. Worlledge, S. M., Corstairs, K. C. and Dacie, J. V.: Autoimmune Hemolytic Anemia Associated With α-Methyldopa Therapy. Lancet 1:135, 1966.

38. LoBuglio, A. F. and Jandl, J. H.: Nature of the Methyldopa Red-Cell Antibody. NEJM 276:658, 1967.

Aldomet (alpha-methyldopa) is the prototype of this reaction. Here the drug cannot be found on the surface of the cell. Gammaglobulin is found and blood group type specificity of the antibody is usually identified (especially anti-e or D, occasionally C). The positive Coombs' can persist for as long as 6 months after discontinuing the drug.

In England the incidence of positive Coombs' reported at 20% level. In the U. S. this figure is considerably lower. Dose relationship does exist.

4. Presence of "Fixed" Normal Nonantibody Globulin on Red Cell Surface:

39. Molthan, L., Reidenberg, M. M. and Eichman, M. F.: Positive Direct Coombs' Tests Due to Cephalathin. NEJM 277:123, 1967.

Cephalathin is the prototype of this reaction. Here the drug binds pre-existing plasma proteins to the red cell surface and agglutination occurs when antiglobulin (Coombs') sera is added. No antibody can be eluted from the cell surface. The higher the concentration of drug the greater the effect. 75% of patients developed a positive Coombs' test. Most were on 4.0 gm/d. Evidence of true increased red cell destruction by this phenomenon is in question, although the clinician is faced with the interpretation of a positive Coombs' frequently reported in an anemic patient during typing and crossing. The Coombs' may become positive by day 4 of therapy and may persist up to 2 months following cessation of therapy.

5. Review and Misc. Mechanisms:

40. Croft, J. D., Swisher, S. N., Gilliland, B. C., Bakemeir, R., Leddy, J. P. and Weed, R. I.: Coombs' -Test Positivity Induced By Drugs: Mechanisms of Immunologic Reactions and Red Cell Destruction. Ann. Int. Med. 68:176, 1968.

D. Neoplasm Associated:

1. Malignant Disease of Lymphoid Tissue:

This is clearly the most common "secondary" form of AIHA. Although a massive literature exists, the mechanism for the sensitization is unproven. Important interrelationships have however been identified:

41. Brody, J. I. and Finch, S. C.: Serum Factors of Acquired Hemolytic Anemia In Leukemia and Lymphoma. JCI 40:181, 1961.

Using the immune adherence technique they studied the abnormal serum factors with acquired hemolytic anemia and leukemia and lymphoma to see if they could be characterized as "antibody". In essence the test is: In the presence of antigen, specific antibody, complement and indicator (primate red cells), the antigen should adhere to the indicator particles. Unfortunately the technique has limitations since it identifies 19S or complement-fixing agglutinins.

They were unable to demonstrate immune adherence with serum from their 7 patients reacted against their own or normal erythrocytes, suggesting that a specific antigen-antibody reaction did not occur and that erythrocyte antibody was not present despite positive direct and indirect Coombs' antiglobulin reactions. This suggests that the serum factors are not the result of antigenic stimulation by red cells.

Subsequent studies did suggest that the red cell and the lymphocyte contain antigenic structures common to both, and that lymphocyte antibody has great avidity for the erythrocyte:

42. Brody, J. I. and Beizer, L. H.: The Cross-Reactivity of Lymphocyte and Red Cell Antibodies. J. Lab. and Clin. Med. 63:819, 1964.

43. Brody, J. I. and Oski, F.: Immunologic Memory of the Normal and Leukemic Lymphocyte. Ann. Int. Med. 67:573, 1967.

The "immune indolence" of the neoplastic lymphocyte has been widely quoted. The presence of AIHA in malignant disease of the lymphocytic family is an apparent paradox. They demonstrated that the "indolence" related only to contact with new antigens (like Salmonella); their response to previously met antigens (like E. coli) was unimpaired.

2. Colloid or Pseudomucin Producing Neoplasms:

The molecular composition of this material yields antigenic similarities to blood group substances A, B, H and Le^a and are in essence the substances produced by "secretors".

44. Landaw, S. A.: Hemolytic Anemia As a Complication of Carcinoma. J. Mt. Sinai Hosp. 31:167, 1964.

45. Tishkoff, G. H. Erythrocyte Mucoids In Acquired Autoimmune Hemolytic Anemia. Blood 28:229, 1966.

3. Misc. Neoplasms:

46. Maldonado, N. I. et al.: AIHA in Chronic Granulocytic Leukemia. Blood 30:518, 1967.

Only scattered cases have been identified with underlying myeloproliferative disorders.

E. Associated With Connective Tissue and/or Immunologic Syndromes:

A large literature has developed concerning the presence and postulated mechanism for AIHA in other "autoimmune" diseases.

47. Schwartz, R. S. and Costen, N.: Autoimmune Hemolytic Anemia: Clinical Correlations and Biological Implications. Seminars In Heme. 3:2, 1966.

48. Halperin, I. C., Menozue, W. F. and Zacharias, P. K.: Autoimmune Hemolytic Anemia and Myasthenia Gravis. NEJM 275:663, 1966.

49. Cohen, S. M. and Waxman, S.: Myasthenia Gravis, CLL and AIHA. Arch. Int. Med. 120, 1967.

A man with a 30 year history of severe myasthenia subsequently developing CLL and AIHA as well as hypogammaglobulinemia. This relationship raises the question of thymic adequacy or function as a predisposition to the course of his disease.

50. Scholler, J., Ching, Y., Williams, C. P., Doves, S. D., Lagunoff, D. and Wedgewood, R. J.: Hyperglobulinemia, Antibody Deficiency, AIHA and Nephritis In An Infant With a Familial Lymphopenic Defect. Lancet 2:825, 1966.

51. Shapiro, M.: Familial Autohemolytic Anemia and Runting Syndrome With Rh_o-Specificity Autoantibody. Transfusion 7:281, 1967.

F. Uncertain Associations and Mechanisms:

1. Experimental:

52. Muirhead, E. E., Groves, M. and Bryan, S.: Positive Direct Coombs' Test Induced by Phenylhydrazine. JCI 33:1700, 1954.

53. Murphy, J. R.: Erythrocyte Metabolism. VI. Cell Shape and the Localization of Cholesterol In the Erythrocyte Membrane. J. Lab. and Clin. Med. 65:756, 1965.

2. Clinical:

54. Selwyn, J. G. and Alexander, S. S.: Positive Coombs' Reaction In Pernicious Anemia. Brit. Med. J. 1:564, 1951.

55. Fudenberg, H. and Rosenfeld, R. E.: Acute Intermittent Porphyria With Transient Auto-immunization. J. Mt. Sinai Hosp. 23:154, 1956.

56. Pirofsky, B., Sutherland, D. W., Starr, A. and Griswald, H. E.: Hemolytic Anemia Complicating Aortic Valve Surgery. NEJM 272:235, 1965.

In presenting 7 of their cases of aortic valve replacement, they identified positive anti-globulin tests in 6 of 7 cases. Of interest these 7 were from over 100 valve replacements. All had had extensive transfusions at surgery (12-27 units). Serologic identification suggested development of anti-e (hr) but that data is not clear and most had a paragglutinin. 4 of the 7 did however have some response to steroids.

The proposed mechanism is modification of the erythrocyte surface antigenic state through turbulent blood flow, with development of a cross reacting erythrocyte autoantibody.

It seems quite clear to most that these are in the main isoimmunization responses as noted above. Even Pirofsky is now willing to concur, with only faint reservations:

57. Pirofsky, B.: Hemolysis in Valvular Heart Disease. Ann. Int. Med. 65:373, 1966.

Etiology of Autoimmune Hemolytic Anemia:

1. Hemolysis is related to alteration of the red cell surface structures by certain injurious agents. (Virus, bacterial enzyme, drugs, chemicals).

2. Antibodies are formed against normal red cells as a result of an aberration of certain antibody-producing clones.

Other Experimental Observations:

Experimental data has added credibility to the clinical observations that alterations in the lymphoid mass serve as the most common etiologic mechanism.

58. Hilyer, B. J. and Howie, J. B.: Spontaneous Autoimmune Disease in NZB/BL Mice. Brit. J. Heme. 9:119, 1963.

This model of naturally occurring AIHA was considered due to an "autoimmune" mechanism. It is now clear that this is a virus induced syndrome.

In spite of the etiologic basis further evidence that altering only the lymphocyte population affects the development of AIHA comes from other NZB mice studies:

59. Denman, A. M., Denman, E. J. and Halborow, E. J.: Suppression of Coombs'-Positive Hemolytic Anemia In NZB Mice by Antilymphocyte Globulin. Lancet II:1084, 1967.

ALG must be given early (from 2 months of age) to alter the advent of positive Coombs'. It did not affect established Coombs'-positive hemolytic anemia.

Finally the recent data suggesting a specific immunologic mechanism in the malignant plasma cell dyscrasias suggests a basis for specific immunization that clinically may appear to arise de novo and suggest an "autoimmune" basis.

60. Kabat, E. A.: A Comparison of Invariant Residues In the Variable and Constant Regions of Human K, Human L and Mouse K Bence-Jones Proteins. Proc. Nat. Acad. Sci. 58: 229, 1967.

Presents important data demonstrating that some myeloma proteins react specifically with antigen and possess many of the properties suggesting a specific antibody response:

1.) An IgA myeloma in a patient with hyperlipidemia reacted specifically with α and β human lipoproteins.

2.) An IgG myeloma demonstrated specific antistreptolysin activity in the FAB fragment.

3.) An IgM macroglobulinemia demonstrated specific reactivity to Fc fragment of human IgG.

THERAPY: (References 14-17; 21-22)

I. Adrenal Steroids:

In addition to affecting an antigen-antibody response, it is evident that a non-immunologic effect blocking or altering splenic phagocytic or sequestering activity also exists:

61. Nicol, T. and Bilbey, D. L. J.: The Effect of Various Steroids On the Phagocytic Activity of the Reticuloendothelial System. From Reticuloendothelial Structure and Function. Edit. J. H. Heller. Ronald Press Co., N. Y., p. 301, 1958.

2. Splenectomy:

62. Micheli, F.: Unmittelbare Effekete der Splenektomie bei einem Fall van Erworkenem Hämolytischen Splenomegalesken Ikterus, Typus Hayem-Widol. Wein. Klin. Wchnschr. 24:1269, 1911.

The first report of splenectomy and resultant beneficial response in AIHA.

63. Chertkow, G. and Dacie, J. V.: Results of Splenectomy in AIHA. Brit. J. Heme. 2:237, 1956.

Results in 28 splenectomies. In 12 of 21 with idiopathic form and 2 of 7 with lymphoma the response was "good". They were able to generate no data to predict response to splenectomy. In their collected series of 255 cases, 52% demonstrated a "good response".

3. Heparin Therapy:

64. Hartman, M. M.: Reversal of Serologic Reactions By Heparin: Therapeutic Implications. II Idiopathic Acquired Hemolytic Anemia. Ann. Allergy 22:313, 1964.

Reported a 39 year old man with chronic AIHA treated with heparin. Coombs' became negative. 2 days after discontinuation of heparin (while on prednisone) both direct and indirect Coombs' were positive. On repeat using 100 mgm of heparin S. C. every 12 hours, the direct Coombs' titer fell from 1:128 to 1:16 in 6 hours and was negative at 7 and 14 days. Again two days after cessation of therapy the direct Coombs' was positive.

65. McFarland, W., Galbraith, R. G. and Miale, A.: Heparin Therapy In AIHA. Blood 15:741, 1960.

Report of a patient who had failed to respond to steroids and splenectomy but did respond dramatically to heparin (150 mgm/day S.C.).

Mechanism of action is not known.

4. Immunosuppression:

66. Schwartz, R. and Dameshek, William: Treatment of Autoimmune Hemolytic Anemia With 6 Mercaptopurine and Thioguanine. Blood 19:483, 1962.

14 patients were treated with thiopurunes (2.5 mgm/kg). 9 patients responded; 5 failures. 9 of the 14 had previously failed to respond to corticosteroid therapy and 4 of these had "good" response to immunosuppressive therapy.

67. Swanson, M. A. and Schwartz, R. A.: Immunosuppressive Therapy. NEJM 277:163, 1967.

5. Miscellaneous:

68. Crosby, W. H. and Rappaport, H.: Reticulocytopenia In Autoimmune Hemolytic Anemia. Blood 11:929, 1956.