Cold Hemagglutination in Acute and Chronic Hemolytic Syndromes

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With the technical assistance of Estelle Downer and Jean Hinz

Cold HEMAGGLUTINATION refers to clumping of red cells whenever a mixture of serum or plasma and erythrocytes, independent of type, is subjected to refrigerator temperature (3 C.). These antibodies may show activity at room temperature (20 C.) in direct relationship to their concentration. This reaction is completely and rapidly reversible at 37 C.

The in vitro phenomenon of cold hemagglutination was described by Landsteiner in 1908. Clough and Richter demonstrated antibodies that were active at reduced temperatures in a case of pneumonia in 1918. Until recent years the presence of cold hemagglutination was considered to be simply an interesting laboratory phenomenon, of more academic than practical interest. In 1943 its diagnostic value in atypical virus pneumonia was recognized by independent groups of investigators. In an excellent review of the subject Stats and Wasserman pointed out that cold hemagglutinins were present in such unrelated conditions as cirrhosis of the liver, pregnancy, the leukemias, tropical eosinophilia, yaws, and occasionally in apparently normal individuals. Subsequent experience has shown that these agglutinins might be of pathogenetic importance in certain types of occlusive arterial disease.

Cold hemagglutinins were first noted to be associated with hemolytic anemia in 1925 when Alexander and Thompson described their presence in leukemia. Since that date, the combination of hemolytic anemia, cold hemagglutination and the Raynaud phenomenon has been made the subject of an increasing number of studies. Despite some uncertainty with regard to the etiologic relationship of hemolysis to cold hemagglutination, there can be little doubt that one phenomenon can at least be associated with the other in a clinically identifiable syndrome.

This report has two objectives. The first is to confirm the characteristic clinical picture of hemolytic anemia attributed to these peculiar antibodies. Secondly, we would like to offer additional data to show that accelerated destruction of erythrocytes in the presence of cold hemagglutinins is not only associated with...
an increase in the mechanical fragility of the agglutinated red cells at low temperatures, but may also be the result of a second antibody which together with complement is capable of causing hemolysis.

**Material and Methods**

Three cases of auto-immune hemolytic disease associated with cold hemagglutinins were studied over a period of four years. One was acute and two were chronic. Blood counts were done at frequent intervals, using standard technics. Hemagglutinins were sought in the patients' heat inactivated (56 C.) serum, diluted serially with saline or 20 per cent bovine albumin, and tested against the patients' (autogenous) and group O Rh negative erythrocytes. Test cells were used as a 2 per cent suspension in isotonic saline or bovine albumin. Trypsinized erythrocytes in saline suspension were employed according to Rosenthal et al. After incubation for four hours at 3 C., 20 C., and 37 C., the preparations were read for agglutination. Agglutination of erythrocytes into one large clump was designated as 4+, into two or three smaller clumps as 3+, into 10 or 15 coarse aggregates as 2+, into fine agglutination as 1+, as a granular suspension ±, and no agglutination was considered negative. Titration for hemolysins using autogenous and group O untreated and trypsinized erythrocytes was accomplished by diluting the patients' serum serially in fresh human type AB Rh negative serum which also served as source of complement. In some experiments, acidified sera were used. The Donath-Landsteiner test as modified by Ham was performed, utilizing normal and trypsinized test erythrocytes. Fresh guinea pig serum, diluted 1 to 30, was employed as complement, and the patients' serum, unless otherwise noted, was fresh. The anti-globulin test was performed in duplicate on washed erythrocytes from blood which was allowed to clot at 37 and 3 C. The anti-globulin serum inhibition test was performed as outlined by Dacie, in which a 2 per cent saline suspension of the patients' washed erythrocytes was added to equal amounts of serially diluted 4 per cent human gamma globulin and anti-globulin serum. The end point of this reaction was the first tube in which agglutination appeared, or the strongest dilution of human gamma globulin which effectively inhibited the anti-globulin reaction (table 3).

The osmotic fragility of erythrocytes was quantitatively determined by the method of Suess et al. Mechanical fragility was estimated by the technic of Shen, Castle and Fleming, utilizing a Burrell mechanical shaker. Osmotic and mechanical fragility determinations were repeated on patients' erythrocytes incubated in their own plasma at 3 C. and 37 C. after 24 hours. Determination of the survival time of transfused erythrocytes was accomplished by the method of Young, Platzer and Rafferty. Serum bilirubin was done by the method of Malloy and Evelyn and fecal urobilinogen determinations on 72 hour pooled specimens by the method of Watson.

**Case Reports**

**Case 1 (Acute Hemolytic Anemia; Atypical Virus Pneumonitis)**

*Clinical Data.* James C. (MCGH 375880), a previously healthy, 52 year old Negro, developed coryza and nonproductive cough two weeks prior to admission. One week later he developed anorexia, progressive weakness and increasing dyspnea. He soon became bedridden because of profound debility. There was no recollection of passing red urine. On physical examination the patient appeared acutely ill, dyspneic and toxic. The temperature was 101.8 F. The sclerae were slightly icteric. There was dullness in both lower lung fields with moist, crepitant rales bilaterally. The heart was rapid and regular. A systolic murmur was heard at the apex. The liver and spleen were not palpable.

*Laboratory Data.* The urine was negative for bilirubin, hemoglobin and hemosiderin. The patient's erythrocytes agglutinated in Hayem's solution at room temperature. After short incubation at 37 C. the clumps became "unloosened" allowing enumeration of the red cells in a warm counting chamber. The hemoglobin was 6.3 Gm. per 100 cubic centimeters. The red cell count was 2.34 M. and the leukocytes 11,500 per cu. mm. with segmented neutrophils 12 per cent, band forms 52 per cent, metamyelocytes 1 per cent, eosinophils 1 per cent,
lymphocytes 25 per cent, and monocytes 9 per cent. Five normoblasts were noted in counting 100 white cells. There was pronounced toxic granulation and polychromatophilia. Erythrophagocytosis by monocytes was noted on the direct peripheral smear. The platelets numbered 356,000 per cu. mm. and reticulocytes 10 per cent. The bone marrow aspirate showed marked normoblastic hyperplasia and a moderate left shift of the granulocytic series. The osmotic fragility test was normal. A sickle cell preparation was negative at the end of 24 hours. The patient's serum agglutinated compatible erythrocytes at 3°C in a dilution of 1 to 4096 when saline and 20 per cent bovine albumin were used as suspending and diluting agents (table 1). Shaking the cold agglutinated erythrocytes on a mechanical shaker produced gross hemolysis. The quantity of hemoglobin liberated by this procedure was not measured. The antiglobulin test was negative. Search for hemolysins by the Donath-Landsteiner, acid hemolysis and heat resistance tests were all negative. Roentgenograms of the chest disclosed increased hilar shadows with diffuse pneumonitis.

Course. (fig. 1). The patient was kept in a room constantly warmed to 30°C. He was given four blood transfusions shortly after admission to the hospital. Treatment with penicillin, 1,400,000 units and aureomycin 1 gram daily, in divided doses, was instituted. The abnormalities in the chest noted on physical and Roentgenographic examination gradually cleared as the patient became afebrile. The hemoglobin and erythrocyte count increased as the
reticulocyte count and cold agglutinin titer declined. All were restored to normal in three weeks. Although in the beginning of this man's illness there was numbness, tingling and cyanosis of the fingers, there were no further signs of Raynaud's phenomenon after he was placed in the warm room.

The patient was discharged 28 days after admission; he was free of symptoms and completely normal in every respect.

Case 2 (Chronic Hemolytic Anemia; Etiology Undetermined)

Clinical Data. Edwin S. (MCGH 423360), an 80 year old white man was first seen in the spring of 1951 because of cardiac decompensation. No hematologic abnormality was detected and he was discharged after digitalization. In November, 1951, he was admitted because of dyspnea, vertigo and numbness and blueness of the hands and exposed parts. There was no icterus and no splenomegaly. Incubation at 37 C. was required to count the erythrocytes because of autoagglutination. They totaled 2,920,000 per cubic millimeter. Cold agglutinins in saline dilution were present in a titer of 1 to 16,000. The patient was transfused twice and discharged. In October, 1952, he was readmitted complaining again of numbness and blueness of his hands. He developed a painful swelling on the medial aspect of his right ankle. Attempted self treatment at home with cold packs led to increase in pain and the swelling on the leg became fluctuant. The patient denied hemoglobinuria.

Physical examination disclosed a thin, pale, elderly man. He was slightly icteric. There was a loud precordial systolic murmur. A lymph node in the left axilla measured 1 centimeter in diameter and was freely movable. The liver edge was felt 4 centimeters below the costal margin but the spleen was not felt. The right ankle was swollen.

Laboratory Data. The hemoglobin was 9 grams per cent, the red count 2,500,000, white count 9,100, and platelets 183,000 per cubic millimeter. The reticulocytes were 5.4 per cent. The smear showed segmented neutrophils 43 per cent, stab forms 24 per cent, lymphocytes 26 per cent, monocytes 6 per cent and eosinophils 1 per cent. Slight polychromatophilia was present but no spherocytes were seen. The bone marrow showed marked normoblastic hyperplasia. The serum bilirubin revealed 0.2 milligrams of direct and 2.0 milligrams of indirect reacting bilirubin. The average of a four-day fecal urobilinogen output showed an excretion of 1,775 milligrams daily, while a 24 hour urine specimen contained 3.1 milligrams of urobilinogen. The N.P.N. was 52 milligrams per cent. An osmotic fragility test showed beginning hemolysis at 0.44 and complete hemolysis at 0.36 per cent saline. Survival of transfused erythrocytes showed disappearance in 30 days in an exponential fashion (fig. 2).

Fig. 2.—Case 2. Survival of transfused erythrocytes before and during treatment.
FIG. 3.—Case 2. Relation of therapy to changes in titer of cold hemagglutinins done by various technics. Patient's serum was diluted with saline or 20 per cent bovine albumin. Washed group "O" Rh negative erythrocytes in saline or albumin dilution were used, fresh or trypsinized. Note prozone phenomenon in albumin dilution. Dotted lines indicate equivocal (±) agglutination.

Serological examination for red cell hemagglutinins was performed shortly after admission to the hospital and was repeated at regular intervals throughout the patient's entire course (fig. 3). A typical profile is reported in detail in table 1. The Coombs' antiglobulin reaction was negative when the patient's blood was allowed to clot at 37 C., but was positive when clotting took place at 3 C., despite subsequent washings with warm saline and incubation at body temperature. The serum was not examined for hemolysins during his first admission.

Cryoglobulins were sought by allowing a small portion of the patient's serum to remain at 3 C. over several hours, but no abnormal jelling was detected.

Examination of the patient's serum for antibodies against the influenza and Newcastle Disease viruses failed to indicate any antibodies in significant titer.*

Biopsy of a lymph node in the left axilla revealed chronic lymphadenitis. Roentgen examination of the chest failed to disclose any abnormal consolidation or pulmonary infiltration.

Course. (figs. 2 and 3). Adrenocorticotropic hormone (ACTH) 80 units in divided doses, was administered intramuscularly daily. Although the patient was markedly improved subjectively, the erythrocyte count varied between 2,500,000 and 2,900,000, the reticulocytes fell to normal limits and the titer of cold agglutinins remained unchanged, except for minor fluctuations in agglutinins demonstrable at room temperature (fig. 3). Before discharge to the out-patient department, medication was changed to cortisone, 75 milligrams daily, but he took medication sporadically or not at all. About a month later, he was seen at the clinic complaining of extreme weakness, dyspnea and generalized pruritis. On examination there was no visible skin eruption. The patient was icteric and pale. There was a fluctuant cyst in the left axilla. The lungs were clear, the heart rapid and regular, and a high pitched apical systolic murmur was present. The liver was palpable 4 centimeters below the

* We wish to express our gratitude to Cdr. John R. Seal, M.C., U.S.N. Great Lakes, Illinois, who examined the serum for influenza virus antibodies; and to Dr. A. S. Evans, University of Wisconsin and Dr. J. O. Alberts, University of Illinois, who performed Newcastle Disease virus titrations.
TABLE 2.—Effect of Varying Dilutions of Human Gamma Globulin on Inhibition of Agglutination of Patient's Erythrocytes by Anti-Globulin (Coombs') Serum by Dacie's Technique

(Each tube contains serially diluted 4 per cent gamma globulin, 0.1 cc., 2 per cent patient's erythrocyte suspension (washed 8X)—0.1 cc. and anti-globulin serum 0.1 cc.)

<table>
<thead>
<tr>
<th>Initial case No.</th>
<th>Dilutions of 4% human gamma globulin</th>
<th>Control (saline)</th>
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<tbody>
<tr>
<td></td>
<td>1:1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1:2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1:4</td>
<td></td>
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<tr>
<td></td>
<td>1:1024</td>
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</tr>
<tr>
<td></td>
<td>1:2048</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1:4096</td>
<td></td>
</tr>
<tr>
<td>2. E. S.</td>
<td>±</td>
<td>±</td>
</tr>
<tr>
<td>3. E. B.</td>
<td>1+</td>
<td>1+</td>
</tr>
<tr>
<td>4. I. B.*</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

* Auto-immune (warm) hemolytic anemia. Included for comparison.

costal margin, but the spleen was not felt. The patient was admitted to the hospital for further study.

Further laboratory study showed a hemoglobin of 6 grams per cent. The erythrocytes and leukocytes numbered 1,670,000 and 10,550 per cubic millimeter, respectively. Differential count showed segmented neutrophils 54 per cent, stabs 20 per cent, myelocytes 1 per cent, metamyelocytes 1 per cent, eosinophils 4 per cent, basophils 3 per cent, lymphocytes 11 per cent and monocytes 6 per cent. Polychromatophilia and spherocytosis were noted. The platelets numbered 316,000 per cubic millimeter and the reticulocytes 0.12 per cent. The serum bilirubin was 1.6 milligrams per 100 cubic centimeters, of which 1.4 milligrams were indirect. Osmotic fragility of erythrocytes showed beginning hemolysis at 0.44 and complete hemolysis at 0.38 per cent saline. Mechanical fragility resulted in a 28 per cent liberation of hemoglobin. A 24 hour urine specimen contained 7.2 milligrams of urobilinogen but was negative for hemosiderin or free hemoglobin.

On this occasion the titer of cold antibodies, which had not decreased appreciably under the influence of cortisone, showed a marked increase now that the drug had been discontinued. Cold isoantibodies (3 C.) were present in dilutions of 1 to 2048 (saline), 1 to 64,556 (trypsin) and 1 to 4096 (albumin). At 20 C., saline, trypsin and albumin agglutinins were present in dilutions of 1 to 1, 1 to 128 and 1 to 16 respectively. As before, there was no agglutination at 37 C. The direct Coombs' test was strongly positive, even after the blood was allowed to clot at body temperature and was washed with warm saline. The Coombs' reaction was not inhibited by the addition of serially diluted gamma globulin solution (table 2).

An additional serologic peculiarity was discovered when a Donath-Landsteiner test was performed (table 3). Hemolysis occurred within 60 minutes after the patient's fresh serum and guinea pig complement (diluted 1 to 30) were incubated against a fresh saline suspension of group O Rh negative erythrocytes. Curiously, the hemolytic reaction did not take place when a saline suspension of the patient's untreated erythrocytes was employed. On the other hand, hemolysis occurred promptly when the test autogenous and normal Group "O" erythrocytes were trypsinized. Hemolysis was not noted in the absence of complement, when heat inactivated serum was employed. Because the hemolytic reaction was immediate and did not require preliminary cold incubation, this was considered a negative Donath-Landstetter reaction. Further studies showed that the hemolytic reaction occurred when fresh compatible Rh negative red cells were used, when the autogenous test erythrocytes were trypsinized or when the patient's serum was acidified to pH 6.7 with 0.1 N HCl. Seven days after treatment with A.C.T.H. was resumed, titration of hemolysin was negative with fresh erythrocytes, but hemolysis took place in a dilution of 1 to 32 with trypsinized test cells.

Further Course. It was obvious that the patient was in severe hemolytic relapse following self-discontinuance of medication. Accordingly, treatment with A.C.T.H., 80 units intramuscularly, daily in divided doses was reinstituted. Cortisone therapy was started two days.
We are indebted to Doctors Louis H. Sennett and Robert S. Haukohl for permission to study and report this case.

### Table 3.—Hemolysin Determinations

<table>
<thead>
<tr>
<th>Tube no.</th>
<th>Trypsin R.B.C.</th>
<th>Fresh serum 0.2 ml.</th>
<th>Fresh G.P. serum (1:30) 0.1 ml.</th>
<th>Inactivated serum 0.2 ml.</th>
<th>Hemolysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Pt.</td>
<td>Pt. +</td>
<td>0</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>Con.</td>
<td>Pt. +</td>
<td>0</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>Pt.</td>
<td>Con. +</td>
<td>0</td>
<td>0</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>Con.</td>
<td>Con. +</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>Pt.</td>
<td>0</td>
<td>0</td>
<td>Pt.</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>Con.</td>
<td>0</td>
<td>0</td>
<td>Pt.</td>
<td>0</td>
</tr>
<tr>
<td>7</td>
<td>Pt.</td>
<td>0</td>
<td>0</td>
<td>Con.</td>
<td>0</td>
</tr>
<tr>
<td>8</td>
<td>Con.</td>
<td>0</td>
<td>0</td>
<td>Con.</td>
<td>0</td>
</tr>
<tr>
<td>9</td>
<td>Pt.</td>
<td>0</td>
<td>+</td>
<td>0</td>
<td>–</td>
</tr>
<tr>
<td>10</td>
<td>Con.</td>
<td>0</td>
<td>+</td>
<td>0</td>
<td>–</td>
</tr>
</tbody>
</table>

later with an initial dose of 100 mg. daily. The amount was gradually diminished over a period of 18 days to an eventual maintenance dose of 25 milligrams daily. On this regimen, dyspnea diminished, he became ambulatory and was able to perform his usual activities without undue fatigue. The icterus disappeared, the cardiac rate diminished, but the liver remained palpable. Intermittent edema of the ankles was present, which responded favorably to restriction of salt and mercurials. The erythrocyte count became stabilized to a mean of about 2,900,000. The patient was able to tolerate this degree of anemia very well, and did not require blood transfusions. A second erythrocyte survival study which was done during this therapeutic period (fig. 2) showed transfused cells to be present 90 days after they were given, with a linear disappearance curve. The fecal urobilinogen output fell to 300 milligrams daily.

The titer of cold agglutinins showed fluctuations described previously and these antibodies did not disappear entirely during the course of therapy (fig. 3). Two weeks following sustained hormone therapy, the hemolysin disappeared and could no longer be demonstrated. Antibodies in albumin dilution underwent an interesting transformation at the same time. These antibodies could not be demonstrated at low dilutions, but made their appearance in a titer of 1 to 64 and disappeared at 1 to 512, so that a definite prozone phenomenon was demonstrated. The patient's erythrocytes continued to be agglutinated by antoglobulin serum to the present writing. During this therapeutic period the mechanical fragility of freshly drawn erythrocytes was 6 per cent. Following 24 hours incubation at various temperatures, the mechanical fragility became 49 per cent (3 C.) and 41 per cent (37 C.) (fig. 4). The osmotic fragility which showed beginning hemolysis at .46 and complete hemolysis at .30 per cent saline, showed a marked shift to the left after 24 hour incubation at 37 C. (fig. 5).

Thus far, the patient has been maintained on a daily dose of 50 milligrams of cortisone. The hemolytic process appears to be well compensated and though evidences of hemolysis still exist, blood regeneration appears to be able to keep up with the excessive destruction.

### Case 3* (“Cold” Auto-immune Hemolytic Anemia. Etiology Undetermined)

Clinical Data. Estelle B. (Deaconess Hospital #1681), a 67 year old white nurse complained of a persistent "cold" with a nonproductive cough in December, 1953. Although she did not recover from these symptoms, she did not seek medical attention. Raynaud's phenomenon did not develop at any time. In January, 1954 she complained of anorexia, vertigo and easy fatigability. Because these symptoms increased in severity she was admitted to the hospital on February 26, 1954. On physical examination this woman was pale and chronically ill. Icterus was not noted. There were no palpable lymph nodes. The blood pressure was 180/80. A grade II systolic murmur was heard at the apex. The liver and spleen

* We are indebted to Doctors Louis H. Sennett and Robert S. Haukohl for permission to study and report this case.
wet were not palpable and no masses were present in the abdomen. The extremities disclosed no

cyanosis or any other abnormalities.

Laboratory Data. The hemoglobin was 9 grams, erythrocyte count 2,900,000, leukocyte
count 7,000, and reticulocytes 3.3 per cent. There were 48 per cent segmented neutrophils, 3
per cent stabs, 38 per cent lymphocytes, 9 per cent monocytes, and 2 per cent eosinophils.
There was slight polychromatophilia but no spherocytes were seen. The sedimentation rate
was 42 millimeters in one hour. The total serum bilirubin concentration was 1.1 milligrams
per 100 milliliters. Bone marrow examination disclosed normoblastic hyperplasia. There
was no free hemoglobin in the urine. Roentgen study of the chest, abdomen and entire
gastrointestinal tract disclosed no abnormalities.

Serologic Study. Serologic study was performed by Doctor T. J. Greenwalt one day prior
to administration of cortisone, and in our laboratory two weeks following institution of
treatment. Results were similar from both laboratories. At 3 C. agglutinins for “O” negative
cells were present in a titer of 1 to 32 (saline), 1 to 2048 (albumin) and 1 to 1024 (trypsin)
table 1). The antiglobulin reaction was 2+ positive. Erythrocyte coating antibodies were
not neutralized by 4 per cent human gamma globulin (table 2). Search for hemolysins dis-
closed the presence of a hemolytic antibody in a titer of 1 to 32. Hemolysins were demon-
strated against trypsinized erythrocytes after 90 minutes at 37 C. The mixture of a 3 per

![Graph](image-url)

**Fig. 4.**—Changes in mechanical fragility after cold and warm incubation

![Graph](image-url)

**Fig. 5.—** Effect of warm incubation (37 C.) upon the osmotic fragility curve. There was
no increase in osmotic fragility following incubation at 3 C.
cent saline suspension of the patient's washed erythrocytes with fresh guinea pig complement (1:30) or fresh human AB serum resulted in immediate hemolysis. Hemolysis did not occur when heat inactivated serum was used. This suggested the presence of a hemolytic antibody directly attached to her erythrocytes (table 3).

The effect of cold and warm incubation upon the mechanical and osmotic fragility is illustrated in figures 4 and 5. Although cold had no effect upon the osmotic fragility, there was a marked increase in mechanical fragility following 24 hours incubation at 3 C. The mechanical fragility could not be done after 24 hours incubation at 37 C. because the erythrocytes were partially hemolyzed. The marked left shift in osmotic fragility manifested after 24 hours incubation at 37 C. was the result of its hemolytic reaction.

Course. Therapy was initiated with cortisone, 300 milligrams daily, gradually reduced to 50 milligrams daily. After seven months of continuous treatment, there has been but slight improvement in the red cell count which has been maintained between 2.58 and 3.71 M. There has been no change in the concentration of cold hemagglutinins and the antiglobulin reaction is still positive. Hemolysins are still demonstrable in the serum in a dilution of 1 to 2, and can still be demonstrated on the surface of the patient's erythrocytes by the addition of complement.

**COMMENTS**

*Clinical Aspects.* Comparison of our cases with 26 similar patients previously reported in the literature strongly suggests a characteristic clinical and serologic picture (table 4).

Our reported cases exemplify the course which may be assumed by the acute or chronic form of hemolytic anemia due to cold agglutinins. The first patient's disease was characterized by sudden hemolysis, rapid development and self-limited course with complete recovery from both pneumonia and hemolytic anemia coinciding with disappearance of cold antibodies.

On the other hand, the disease of the second and third patients was insidious in onset, of a chronic nature and was progressive in the absence of therapy. Chronic auto-immune hemolytic anemia associated with cold agglutinins is seen predominantly in older men. Frequently there is no demonstrable etiologic factor in the chronic group, but this syndrome has been noted in leukemia, malaria, cirrhosis of the liver and hereditary spherocytosis. These relationships have been rare and inconstant.

The combination of acute hemolytic anemia associated with cold agglutinins with virus pneumonia and the observation that certain viruses have the property of agglutinating or even hemolyzing erythrocytes in vitro has led to numerous speculations, which have become accelerated following the announcement by Moolten and Clark of the isolation of the Newcastle Disease virus from their case of acute hemolytic anemia. The speculation that erythrocytes subjected to the influence of virus may become "auto-antigenic" is intriguing but awaits confirmation. Antibodies for the Newcastle Disease virus could not be demonstrated in our second case, nor in members of the immediate family, nor was there an increase in the influenza virus antibody titer. No virus studies were carried out in cases 1 and 3, although a virus infection was suspected at some time during their illness.

Raynaud's Phenomenon. Raynaud's phenomenon is a common clinical feature of the syndrome caused by cold hemagglutination, and occasionally, has led to gangrene. Although this sign is present in most chronic cases, there are instances where the Raynaud phenomenon is not encountered during the acute
### Table 4.—Clinical Data. Previously Reported Cases of Hemolytic Anemia and Cold Hemagglutination

<table>
<thead>
<tr>
<th>Authors—year</th>
<th>Age</th>
<th>Sex</th>
<th>Duration</th>
<th>Associated disease</th>
<th>Raynaud's syndrome</th>
<th>Splenomegaly</th>
<th>Cold agglutinin dilution of serum</th>
<th>Hemolysin</th>
<th>Coomb's test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alexander &amp; Thompson 1925—1926</td>
<td>48</td>
<td>M</td>
<td>3 yrs.</td>
<td>Chronic leukemia</td>
<td>Present</td>
<td>“Just palpable”</td>
<td>$&gt;1280$</td>
<td>Not found</td>
<td>—</td>
</tr>
<tr>
<td>Boxwell &amp; Bigger 1931—1931</td>
<td>65</td>
<td>F</td>
<td>6 mos.</td>
<td>Leukemia?</td>
<td>Absent</td>
<td>Absent</td>
<td>Present not titrated</td>
<td>—</td>
<td>—</td>
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<tr>
<td>Roth 1935—1935</td>
<td>59</td>
<td>M</td>
<td>2 yrs.</td>
<td>Unknown</td>
<td>Present</td>
<td>Absent</td>
<td>Present not titrated</td>
<td>Present</td>
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<td>Salem 1935—1935</td>
<td>50</td>
<td>M</td>
<td>4 yrs.</td>
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<td>Present</td>
<td>Absent</td>
<td>1024</td>
<td>Present</td>
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<tr>
<td>Ernstene &amp; Gardner 1935—1935</td>
<td>38</td>
<td>M</td>
<td>2 yrs.</td>
<td>Unknown</td>
<td>Present</td>
<td>Absent</td>
<td>Present not titrated</td>
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<tr>
<td>McCombs &amp; McElroy 1937—1937</td>
<td>57</td>
<td>M</td>
<td>3 yrs.</td>
<td>Unknown</td>
<td>Present</td>
<td>Absent</td>
<td>Present not titrated</td>
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<td>Hans 1938—1938</td>
<td>45</td>
<td>M</td>
<td>2 yrs.</td>
<td>Unknown</td>
<td>Present</td>
<td>Absent</td>
<td>Present not titrated</td>
<td>Not found</td>
<td>—</td>
</tr>
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<td>Sezary, Kipfer &amp; Gharib 1938—1938</td>
<td>60</td>
<td>M</td>
<td>2½ yrs.</td>
<td>Cirrhosis</td>
<td>Present</td>
<td>“18 x 9 Cms.”</td>
<td>Present not titrated</td>
<td>Not found</td>
<td>—</td>
</tr>
<tr>
<td>Dameshek 1943</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Case 1</td>
<td>26</td>
<td>M</td>
<td>1 week</td>
<td>Pneumonia, Rx. sulfonamides</td>
<td>Absent</td>
<td>Absent</td>
<td>512</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Case 2</td>
<td>50</td>
<td>F</td>
<td>1 week</td>
<td>Virus pneumonia, Rx. sulfonamides</td>
<td>Not stated</td>
<td>Absent</td>
<td>Present not titrated</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Stats &amp; Bullowa 1943—1946</td>
<td>64</td>
<td>M</td>
<td>15 yrs.</td>
<td>Unknown</td>
<td>Present</td>
<td>Absent</td>
<td>32,000</td>
<td>Not found</td>
<td>—</td>
</tr>
<tr>
<td>Ginsberg 1946</td>
<td>34</td>
<td>M</td>
<td>1 day</td>
<td>Primary atypical pneumonia</td>
<td>—</td>
<td>Absent</td>
<td>2048</td>
<td>Not found</td>
<td>—</td>
</tr>
<tr>
<td>Whittle, Lyell &amp; Gatman 1947—1949</td>
<td>56</td>
<td>F</td>
<td>5 yrs.</td>
<td>Unknown</td>
<td>Present</td>
<td>Absent</td>
<td>2,000,000</td>
<td>Not found</td>
<td>—</td>
</tr>
<tr>
<td>Malley &amp; Hickey 1949—1949</td>
<td>69</td>
<td>M</td>
<td>4 yrs.</td>
<td>Unknown</td>
<td>Present</td>
<td>Absent</td>
<td>2048</td>
<td>Not found</td>
<td>—</td>
</tr>
<tr>
<td>Colmers &amp; Shavely 1947—1947</td>
<td>21</td>
<td>F</td>
<td>3 days</td>
<td>Primary atypical pneumonia</td>
<td>Present</td>
<td>Not stated</td>
<td>5120</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Bateman 1949</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Case 1</td>
<td>22</td>
<td>M</td>
<td>Few days</td>
<td>Primary atypical pneumonia</td>
<td>Absent</td>
<td>Not stated</td>
<td>2650</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Case 2</td>
<td>Rubin, Jacobson &amp; Meyer 1949</td>
<td>43</td>
<td>M</td>
<td>2 mos.</td>
<td>Unknown</td>
<td>Present</td>
<td>Not stated</td>
<td>2,621,220</td>
<td>1024</td>
</tr>
<tr>
<td>Neely, Baria, Smith &amp; Stone 1951</td>
<td>30</td>
<td>F</td>
<td>8 days</td>
<td>Primary atypical pneumonia</td>
<td>Absent</td>
<td>Absent</td>
<td>4096</td>
<td>Present</td>
<td>—</td>
</tr>
<tr>
<td>Ferriman, Dacie, Koele &amp; Fuller ton 1951</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Case 1</td>
<td>61</td>
<td>F</td>
<td>11 yrs.</td>
<td>Unknown</td>
<td>Present</td>
<td>1 inch</td>
<td>64,000</td>
<td>Present</td>
<td>Positive</td>
</tr>
<tr>
<td>Case 2</td>
<td>78</td>
<td>M</td>
<td>2 yrs.</td>
<td>Thrombophlebitis</td>
<td>Present</td>
<td>Absent</td>
<td>128,000</td>
<td>Present</td>
<td>Positive</td>
</tr>
<tr>
<td>Case 3</td>
<td>67</td>
<td>F</td>
<td>1 yr.</td>
<td>Unknown</td>
<td>Present</td>
<td>Absent</td>
<td>64,000</td>
<td>Present</td>
<td>Positive</td>
</tr>
<tr>
<td>Aaron 1952</td>
<td>46</td>
<td>M</td>
<td>3 days</td>
<td>Primary atypical pneumonia</td>
<td>Present</td>
<td>Absent</td>
<td>4096</td>
<td>Present</td>
<td>Positive</td>
</tr>
<tr>
<td>Moolten &amp; Clark 1952</td>
<td>37</td>
<td>F</td>
<td>1 week</td>
<td>Newcastle virus disease. Disseminated L.E.?</td>
<td>Present</td>
<td>Present size not stated</td>
<td>256</td>
<td>Not stated</td>
<td>—</td>
</tr>
<tr>
<td>Nelson &amp; Marshall 1953</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Case 1</td>
<td>47</td>
<td>M</td>
<td>1 yr.</td>
<td>Unknown</td>
<td>Present</td>
<td>Absent</td>
<td>20,480</td>
<td>Not found</td>
<td>Positive</td>
</tr>
<tr>
<td>Case 2</td>
<td>40</td>
<td>M</td>
<td>2 yrs.</td>
<td>Unknown</td>
<td>Present</td>
<td>Absent</td>
<td>20,480</td>
<td>Not found</td>
<td>Positive</td>
</tr>
<tr>
<td>Moonishlin et al. 1954</td>
<td>36</td>
<td>M</td>
<td>3 days</td>
<td>Primary atypical pneumonia</td>
<td>Present</td>
<td>Not stated</td>
<td>1024</td>
<td>Present</td>
<td>Positive</td>
</tr>
<tr>
<td>Pisciotta 1954</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Case 1</td>
<td>52</td>
<td>M</td>
<td>2 wks.</td>
<td>Primary atypical pneumonia</td>
<td>Present</td>
<td>Absent</td>
<td>4096</td>
<td>Not present</td>
<td>Negative</td>
</tr>
<tr>
<td>Case 2</td>
<td>80</td>
<td>M</td>
<td>3 yrs.</td>
<td>Unknown</td>
<td>Present</td>
<td>Absent</td>
<td>16,000</td>
<td>Present</td>
<td>Positive</td>
</tr>
<tr>
<td>Case 3</td>
<td>67</td>
<td>F</td>
<td>3 mos.</td>
<td>Unknown</td>
<td>Present</td>
<td>Absent</td>
<td>32(saline) 1024(trypsin albumin)</td>
<td>Present</td>
<td>Positive</td>
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</tbody>
</table>
Direct observations of agglutination phenomenon in hemolytic anemia associated with cold hemagglutinins have been made in vivo by study of the conjunctival mucous membrane following application of cold, and by studies of the capillaries of the nail bed. These demonstrated that intravascular hemagglutination resulted in a marked diminution of blood flow. It was shown with plethysmographic and calorimetric techniques that blood flow was totally abolished in cold agglutination, whereas it was actually increased in Raynaud’s (vasospastic) disease. These findings were interpreted as indications that Raynaud’s phenomenon associated with cold hemagglutination in an occlusive, rather than a vasospastic phenomenon.

Splenomegaly. Splenomegaly was not present in any of our patients, nor did it develop at any time during the course of illness. Splenomegaly was said to be present in only five of 27 previously reported cases. In these patients another cause for splenomegaly was usually demonstrable such as chronic granulocytic leukemia, and cirrhosis of the liver. One case had many of the features of the collagen group diseases, and in the two remaining, splenomegaly was unexplained although this organ did not reach a prominent size. From these clinical observations, one gains the impression that lack of splenomegaly may be part of the clinical picture in hemolytic anemia associated with cold agglutinins. Although time may not permit enlargement of the spleen in acute hemolytic anemia, this finding, as well as evidences of intravascular hemolysis, probably serves to minimize the role of this organ in the pathogenesis of hemolysis in the chronic variety. The inconstancy of improvement following splenectomy in these cases supports this hypothesis.

Pathogenetic Aspects of Hemolysis. The mechanism of premature destruction of red cells, when it occurs in the presence of cold antibodies has not been defined. It has been recognized that elevation in titer of cold agglutinins is possible without the occurrence of the hemolytic syndrome. Such a pathologic state may be characterized clinically solely by the presence of Raynaud’s phenomenon in an otherwise asymptomatic individual or hematologically by difficulty in preparing routine red counts, blood smears, blood typing and cross matching because of auto-agglutination at room temperature. In primary atypical pneumonia the titer of cold agglutinins may rise as high as 1:10,000 without the development of hemolysis. These clinical observations in addition to lack of intrinsic lytic activity of cold agglutinins and inability to demonstrate utilization of complement led Stats and Wasserman to doubt the etiologic relationship between hemolytic anemia and cold hemagglutination.

On the other hand, intravascular hemolysis, as evidenced by hemoglobinemia, hemoglobinuria and/or methemalbumin in the plasma, occurring spontaneously or provoked by cold, may characterize the presence of cold agglutinins in high dilution. The importance of these antibodies in the pathogenesis of hemolysis was illustrated by our first case, in whom clinical recovery was attended by a diminution in their titer and eventual disappearance (fig. 1). Conversely, the persistent presence of cold antibodies as in cases 2 and 3 correlated well with continuous hemolysis in these patients.

Hemolysis is a complex phenomenon which probably involves a variety of pathogenetic mechanisms. Stats demonstrated that cold agglutinated erythrocytes were mechanically fragile, and concluded that hemolysis was a result of...
mechanical buffeting sustained by cold agglutinated red cells in vivo. Increased mechanical fragility was demonstrated in all our patients and was quantitatively increased in cases 2 and 3 (fig. 4). That increases in mechanical fragility are not the sole explanation for hemolytic anemia in cold hemagglutination, is emphasized by those instances in which cold agglutinins are present in high titer, but hemolytic anemia does not occur. In such an instance, destruction of erythrocytes may be explained by various lytic substances, either circulating in the peripheral blood or present in the fixed tissues.

The earlier literature pertaining to cold hemagglutination associated with hemolytic anemia indicated that the antibodies in such cases might have hemolytic properties. On the other hand a number of investigators failed to demonstrate a hemolytic antibody, even when it was sought specifically. In many of these instances refined technics such as trypsinization of erythrocytes or acidification of the test medium were not employed. In 1950 Dacie described a cold hemolysin associated with cold agglutinins, when the latter were in high titer. In 1951 Ferriman, Dacie et al. found a similar type of hemolytic antibody in three patients with the cold agglutinin-hemolytic anemia syndrome. This antibody was of fairly broad thermal amplitude and its upper limit of activity was 30°C. It was unusually sensitive to changes in pH and was particularly active if the patient’s serum was acidified to pH 6.5 before the test erythrocytes were added.

In our two patients, the marked left shift in the osmotic fragility curve following warm incubation was probably the effect of hemolysin. It was determined in these patients that hemolysin was active in body temperature. Of special interest in case 3 was the demonstration that hemolysin could coat erythrocytes, and could be demonstrated by the addition of guinea pig and/or human complement to the washed red cells.

The relationship of hemolysins to cold agglutinins is unknown. It may be postulated that the naturally occurring incomplete cold agglutinins may undergo quantitative increase and may even change qualitatively to assume the properties of a hemolysin upon stimulation. The stimulus may be temporary, such as atypical virus pneumonia, or permanent and unknown. The sudden appearance of hemolysin following discontinuance of cortisone therapy in our second patient suggests the rebound phenomenon occasionally seen whenever this hormone is suddenly stopped. Diminished adrenal activity may result in the return of the original disease in more exaggerated fashion, so that a hemolysin may be an expression of exaggerated or altered cold agglutinin formation.

Recent evidence indicates that there may be a number of different types of cold hemagglutinins. The agglutinin demonstrated in saline dilution is considered a simple, bivalent antibody, easily eluted from the erythrocyte surface by change in temperature and for these reasons is not demonstrated by the Coombs’ antiglobulin reaction. In 1950 Dacie found in normal and pathologic sera an antibody which attached itself to erythrocytes after cold incubation in their own serum and was thus demonstrated by antiglobulin serum. This has been referred to as incomplete cold antibody and may be demonstrable as well in bovine albumin medium, or with the use of trypsinized test cells. The latter technic has been particularly useful in the demonstration of this antibody in normal sera frequently in low titer. Studies of the electrophoretic pattern of
serum containing cold agglutinins before and after absorption with erythrocytes have suggested that this antibody has the mobility of gamma globulin. On the other hand more recent studies of the "Coombs" reacting antibody in this disease have shown that this reaction is not inhibited by the addition of purified human gamma globulin to the Coombs' serum. This reaction is apparently peculiar to cold antibodies and suggests that they may be present in a serum fraction other than gamma globulin.

Finally, mention must be made of erythrophagocytosis as contributing to hemolysis to some extent. This phenomenon was noted in our first patient, but not in the other two. It is difficult to conceive how a limited number of circulating and fixed reticuloendothelial cells can dispose of a large enough number of erythrocytes to cause so profound an anemia in so short a time as was manifested in our patient. Nevertheless, the appearance of phagocytic cells in the peripheral blood suggested that erythrophagocytosis might play at least a limited role in production of the anemia.

Summary and Conclusions

1. Three cases have been described of hemolytic anemia associated with autoagglutination of erythrocytes in the cold. These cases were characterized clinically by pallor, slight icterus and lack of splenomegaly. Raynaud's phenomenon was present in two of the three patients. The first patient had an acute self-limited course. The second and possibly the third were chronic and apparently persistent.

2. A hemolysin was demonstrated in the chronic variety of hemolytic anemia. This antibody was active at body temperature, required complement and appeared to be independent of the cold agglutinin. It disappeared following therapy with cortisone. In the third case, hemolysin was demonstrated directly attached to the surface of the patient's erythrocytes by the addition of complement to the washed red cells. The hemolytic reaction was enhanced by the use of trypsinized test erythrocytes.

3. "Complete and incomplete" cold agglutinins were demonstrated by the use of saline, albumin and trypsin techniques as well as by the Coombs' antiglobulin reactions. Erythrocyte coating antibodies were not neutralized with human gamma globulin. Cold agglutinins and erythrocyte coating antibodies were unaffected by cortisone therapy.

4. Survival of transfused erythrocytes was increased in one patient during the period of cortisone treatment, despite the fact that cold agglutinins persisted.

5. Cold agglutinated erythrocytes have increased mechanical fragility when the cold agglutinin is present in high titer. Incubation at 37 C. for 24 hours causes a marked shift to the left in the osmotic fragility curve.

6. Study of the serum in one patient for antibodies to Newcastle virus disease and influenza failed to disclose these antibodies in significant titer.

Summario e Conclusiones in Interlingua

(1) Es describite tres casos de anemia hemolytic acompaniate de autoagglutination frigide de erythrocytos. Characteristicas clinica eseva un leve grado de ictero, pallor, e le absentia de splenomegalia. Le phenomeno de Raynaud eseva
observate in duo del casos. In le prime caso le curso del morbo esseva acute e auto-limitate. In le secunde e possibilmente le tertie caso, le morbo esseva chronic e apparentemente persistente.

(2) Le presentia de un hemolysina esseva demonstrate in le casos de anemia hemolytic chronic. Iste anticorpo esseva active al temperatura corpore. Illo requireva complemento e pareva non depender del agglutinina frigide. Post therapia a cortisona illo dispareva. In le tertie caso le presentia de hemolysina attachate directamente al superficie del erythrocytos esseva demonstrable per medio del addition de complemento al erythrocytos lavate. Le reaction hemolytic esseva promovite per le uso de erythrocytos trypsinisate.

(3) "Complete e incomplete" agglutininas frigide esseva demonstrate per medio de technique salin, a albumina, e a trypsinca etiam per medio del reactiones antiglobulinic de Coombs. Anticorpo investiente erythrocytos non esseva neutralisabile per medio de globulina gamma ab homines. Agglutininas frigide e anticorpo investiente erythrocytos non esseva afficite per cortisona.

(4) In un del patientes le superviventia de transfundite erythrocytos esseva augmentate durante le periodo del tractamento a cortisona—in despecto del facto que le agglutininas frigide persisteva.

(5) Si le agglutininia frigide es presente in alte titro, illo augmente la fragilitate mechanic del erythrocytos. Incubation pro 24 horas a 37 C. causa in le curva del fragilitate osmotic un marcate deviation verso le sinistra.

(6) Le sero de un del patientes esseva studiate in re le presentia de anticorpo al virus del morbo de Newcastle e de influenza. Tal anticorpo non esseva observe in titros significative.

REFERENCES

COLD HEMAGGLUTINATION

DAMESHEK, W., 61

DADÉ, CASTLE, 60

BOURONCLE, STATS, WHEELER, 56

DAMESHEK, W.: Acquired hemolytic anemia. Physiopathology -:


CURTIS, H. R. A., MOURANT, A. E., MARSHALL, 54

DAMESHEK, J. V.: Cold hemagglutinations and cold hemolysis. The hemolysis produced by shaking cold

DADÉ, W. B., HAM, T. H., GOODKIND, J. F., AND WAGLEY, 50

MOOLTEN, PISCIOTTA, WATSON, 48

SHEN, J., MALLOY, 45

DAMESHEK, W.: Observations on the mechanism of hemolytic transfusion reactions occurring without demonstrable hemolysis. Tr. A. Am. Physi-

DADÉ, W. B., HAM, T. H., AND SHEN, S. C.: Observations on the mechanism of hemolytic transfusion reactions occurring without demonstrable hemolysis. Tr. A. Am. Physi-


BOURONCLE, B. A., DODD, M. C., AND WRIGHT, C. S.: A study of cold hemagglutinins for normal and trypsinized red blood cells in the serum of normal individuals and of hemolyt-

Cold Hemagglutination in Acute and Chronic Hemolytic Syndromes

ANTHONY V. PISCIOTTA, ESTELLE DOWNER and JEAN HINZ