The Unitary Nature of “Complete” and “Incomplete” Pathologic Cold Hemagglutinins

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It has been well known for many years that the sera of patients with certain viral infections, acquired hemolytic anemia or other disorders may contain large quantities of proteins capable of directly agglutinating normal human erythrocytes during cold incubation.1,2 These proteins are designated pathologic “complete” cold agglutinins. The direct agglutination they produce is readily reversed by warming.

The coexistence of pathologic “incomplete” cold antibodies was later inferred from the observation that red cells incubated with such cold agglutinating sera, after warming and washing, were no longer directly agglutinable in the cold but could be reagglutinated by an antiglobulin serum.3-5 The interpretation that these findings were indicative of a distinct “incomplete” antibody seems to have derived from an apparent analogy to the “incomplete” antibodies of human blood grouping systems, particularly the Rh system6 in which the existence of separate “complete” (saline) and “incomplete” agglutinins has now become firmly established.7-10

Subsequent investigations5,11,12 have clearly shown that demonstration of the pathologic “incomplete” cold antibody activity, unlike direct cold agglutination, requires the presence of fresh serum, presumably the complement (C’) components, during the incubation. Furthermore, the reaction of antiglobulin sera with red cells sensitized by pathologic “incomplete” cold antibodies in the presence of fresh serum is not inhibited by prior addition of excess human γ-globulin to the antiglobulin sera.5,13,14 This type of erythrocyte coating has therefore been designated “non-γ-globulin” and is believed to represent, at least in part, bound C’ protein.11,12,14-16 The incomplete cold antibody was presumed to be attached to the red cell surface in association with C’ components in such a manner that the antibody itself was unavailable to react with antiserum to γ-globulin.11 The possibility that this form of incomplete antibody may not be γ-globulin was also considered.13,14 Efforts to clarify this point have been hindered by inability to elute the coating proteins in a form permitting detailed characterization.14,17

More recently, on the other hand, Dacie, Crookston and Christenson postulated11,12 that incomplete cold antibodies distinct from the complete cold agglutinins may not really exist; i.e., that the “non-γ-globulin” erythrocyte coat-
ing material may simply result from the interaction of the complete cold agglutinins with C'. In the present study, this hypothesis was tested by the employment of a variety of physicochemical and serologic methods in an effort to separate the "complete" and "incomplete" cold antibody activities of several pathologic sera. Separation was not achieved. These observations support the concept that a single antibody is responsible for both the direct agglutinating and the C'-fixing activities of the pathologic sera. Antibody functions other than agglutination, e.g., hemolysis, were not studied.

Of incidental interest is the demonstration that the cold antibodies of pathologic sera can be clearly distinguished from the incomplete cold antibodies found in most normal sera by differences in their serologic specificities, and that some differences in specificity apparently existed among the pathologic sera.

**Materials and Methods**

**Sera**

The four pathologic sera examined in this study (table 1) were separated at 37 C. and stored under sterile conditions at −20 C. until used. Feller has demonstrated the stability of cold agglutinins for long periods under these conditions. For studies of the role of C', these sera were heated for 30 minutes at 56 C.; fresh normal serum was then added as desired. Heating did not demonstrably alter antibody activity.

The sera of seven healthy donors were the source of "normal" cold antibodies. The titers of these sera were low, none exceeding 1:8 by macroscopic reading.

Incomplete anti-Rh₀ (anti-D) serum was obtained from a hyperimmunized Rh₀ (D) negative donor (serum R²) and from commercial sources.*

Rabbit antisera to human globulins were prepared essentially as described by Vaughan. Anti-whole serum, prepared by immunization with a pool of fresh, whole human serum, gave strongly positive reactions both with red cells sensitized by incomplete anti-Rh₀ antibodies and with red cells sensitized by pathologic or normal incomplete cold antibodies in the presence of complement. Anti-whole serum thus incorporated the specificities of both the "anti-γ-globulin" and the "anti-non-γ-globulin" sera described below. "Anti-γ-globulin" serum, prepared by immunization with highly purified human γ-globulin (Cohn Fr. II₁, Squibb), was capable of agglutinating anti-Rh₀ sensitized red cells but not those sensitized with incomplete cold antibody. "Anti-non-γ-globulin" serum, prepared by absorption of anti-whole serum with optimal amounts of Cohn Fr. II (Squibb, Lot Rework 11-20-54) to precipitate all anti-γ-globulin antibodies, no longer agglutinated anti-Rh₀ sensitized red cells although the reactivity with incomplete cold antibody sensitized cells was retained. All rabbit antisera were thoroughly absorbed with washed human A₁, B, and O erythrocytes prior to use.

The latter two antiglobulin sera might be more correctly designated "anti-γ₂-globulin" and "anti-non-γ₂-globulin" respectively. Antibodies to γ₁-globulin (also designated β₂M and β₂A globulins) may not be completely removed from an anti-whole serum by absorption with Cohn Fr. II²²,²³

**Erythrocytes**

The human erythrocytes employed in all serologic reactions were freshly drawn from normal group O donors on the day of each experiment. Preliminary work showed no signif-

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* A preliminary account of this work has been previously reported.¹⁸

† The participation of Dr. Richard W. Hill in the preparation of these antisera is gratefully acknowledged.
### Table 1.—Clinical Data and Titers on the Pathologic Sera Under Study

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age</th>
<th>Sex</th>
<th>Clinical Diagnosis</th>
<th>Duration of Disease*</th>
<th>Direct Cold Agglutinin Titer</th>
<th>&quot;Incomplete&quot; Cold Antibody Titer</th>
</tr>
</thead>
<tbody>
<tr>
<td>N. P.</td>
<td>73</td>
<td>F</td>
<td>idiopathic chronic AHD</td>
<td>3 yrs.</td>
<td>1:10,240</td>
<td>1:2560</td>
</tr>
<tr>
<td>W. B.</td>
<td>42</td>
<td>F</td>
<td>transient AHD secondary to PAP</td>
<td>1-2 mos.</td>
<td>1:640</td>
<td>1:160</td>
</tr>
<tr>
<td>W. N.</td>
<td>46</td>
<td>M</td>
<td>chronic renal disease, type uncertain</td>
<td>4-5 yrs.</td>
<td>1:640</td>
<td>1:80</td>
</tr>
<tr>
<td>J. F.</td>
<td>19</td>
<td>F</td>
<td>transient AHD secondary to PAP</td>
<td>1 wk.</td>
<td>1:1280</td>
<td>1:320</td>
</tr>
</tbody>
</table>

*At the time serum was obtained for study.

†AHD = "Autoimmune" hemolytic disease.

‡PAP = Primary atypical pneumonia.

The majority of the experiments on pathologic cold antibodies utilized the cells of one O Rh negative donor because his fresh serum consistently lacked demonstrable "normal" cold antibodies and could serve as a source of C' without the risk of misleading effects due to normal cold antibodies.

Human erythrocytes of the "Bombay" type were freshly obtained from a healthy donor previously studied in this laboratory. These erythrocytes were not agglutinated by anti-A or anti-B sera, or by lectins having anti-H specificity (Eulex Europaeus). The donor's other blood groups were: MN, R_f(cDE/cde), K, Le(a-), Fy(a+).

Human I-negative ("i") erythrocytes from an adult donor (J. S.) were very generously supplied by Dr. T. J. Greenwalt, Milwaukee Blood Center, Inc. The other blood groups of J. S. were: O, MNS, P, R_f(cDE/cde), Lu(a-), Kk, Le(a+b-), Fy(a-), Jk(a-).

### Complement

The source of complement (C') in all studies of pathologic cold antibodies was the fresh autologous serum of the red cell donor, used in a dilution, e.g. 1:10, exceeding the titer of the donor's own normal cold antibodies (see comments under Erythrocytes). For experiments involving "Bombay" red cells or I-negative red cells, the fresh serum of a normal group O donor was used because of the "natural" anti-H or anti-I antibodies respectively in the autologous sera.

### Serologic Titration

The diluents employed were: 0.15M saline; and, triethanolamine-buffered saline (TBS), pH 7.3, ionic strength 0.15, containing $1.5 \times 10^{-4}$ M Ca$^{++}$ and $5.0 \times 10^{-4}$ M Mg.$^{++}$

Titration of cold antibodies was carried out as follows. Serial doubling dilutions of antibody were made in saline. In studies requiring careful quantitation, such as the absorption protocols described below, pipettes were changed at each dilution to avoid carry-over. A 3 per cent suspension of normal erythrocytes was prepared either in fresh serum diluted in TBS or in TBS alone. The serum dilutions and the cell suspension were brought to 2 C., mixed, and incubated for one hour at 2 C. The tubes were then centrifuged for one minute at 800 rpm in an International Size 2 refrigerated centrifuge with a radial distance of 20 cm. The direct agglutination was read macroscopically, still at 2 C. The tubes were then placed at 37 C. for 10 minutes and gently agitated, followed by three washings of the red cells with saline at 37 C. Either anti-whole serum or anti-non-γ-globulin serum, usually
in a 1:20 saline dilution,* was added to each tube and the tubes were immediately centri-
fuged at room temperature for one minute at 800 to 1000 rpm. An “immediate” reading of
agglutination was made macroscopically. The tubes were shaken, allowed to stand for 15
minutes, and a second centrifugation and macroscopic reading were carried out. Since the
agglutination was nearly always stronger at 15 minutes, only readings at this interval will
be presented in this paper. For both direct agglutination and agglutination with antiglobulin
sera, the following grading was used: 4+, if all cells were in a tight clump; 1+, if all the
clumps were fine but easily visible macroscopically; 2+ and 3+ reactions were intermediate.
Controls, consisting of a 3 per cent cell suspension in either TBS
or diluted autologous
serum, were handled identically and gave negative results throughout the study.

Titrations of incomplete anti-Rh0 antibodies were determined after 37 C. incubation for
one hour, using an anti-γ-globulin serum.

Electrophoresis

Zone electrophoresis was carried out in a starch block at 4 C., employing a pH 8.6
barbital buffer.25 Evans blue dye was used to locate the albumin. Following the electrophoresis, 1 cm. segments were cut out and the protein in each segment eluted in 9 ml. of
0.15M saline through sintered glass filter tubes. The protein concentration in each fraction
was determined by the Folin and Ciocalteu reaction.20

Ultracentrifugation

Preparative ultracentrifugation in a sucrose density gradient was performed in a Spinco
model L refrigerated centrifuge with a swinging bucket rotor (SW39L), as previously de-
scribed.27 The samples were spun at 35,000 rpm for 13 to 15 hours. After centrifugation,
approximately 0.5 ml. fractions were withdrawn from above through Pasteur pipettes
rigidly clamped to a ringstand and fitted with a screw-type pro-pipette. The celluloid liner
tube containing the centrifuged serum was attached with transparent tape to a microscope
barrel which could be slowly raised to allow penetration of the pipette to any desired depth
under direct vision and with negligible agitation.

Addition of Evans blue dye to the diluted serum sample prior to centrifugation marked
the position of albumin. Addition of a small quantity of incomplete anti-Rh0 serum al-
lowed localization of 7S γ-globulin.7 when the ultracentrifugal fractions were subsequently
incubated at 37 C. with O Rh0 positive red cells and tested with an anti-γ-globulin serum.
The direct cold agglutinin activity itself, detected by testing with O Rh0 negative red
cells, served to identify the 19S γ-globulin region.27 29

Although the high concentration of sucrose in the undiluted fractions caused some inter-
ference with agglutination reactions, this effect was obviated by dilution of the samples
with equal volumes of 0.15M saline. This permitted titration of the various fractions by
simple saline dilutions. Dialysis to remove sucrose prior to titration did not alter the results.

Chromatography

Anion exchange column chromatography using diethylaminoethyl (DEAE) cellulose
was performed according to the method of Peterson and Sober as modified by Fahey and
associates for human serum30 and human γ-globulin.31 A 1.2 cm. O.D. pyrex column was
packed with the adsorbent, under pressure, to a height of 23 cm. Either whole serum or
the concentrated, electrophoretically separated γ-globulins containing the cold agglutinins
were applied to the column with 0.02M phosphate buffer at pH 8.0. Continuous gradient
equilution was accomplished with 0.3M phosphate buffer at pH 4.2 for the fractionation of
whole serum, and with 0.3M phosphate at pH 8.0 for fractionation of the electrophoretically-
ly isolated γ-globulins. Between 150 and 165 ml. of effluent were collected in 3 ml. frac-

*A 1:20 antiglobulin serum dilution was chosen for most titrations because a greater
collection did not yield significantly stronger reactions, whereas a greater dilution
yielded weaker reactions.
tions with a flow rate of 15 ml per hour. The relative protein concentration of each fraction was determined by measurement of the optical density at 280 mμ in a Beckman model DU spectrophotometer.

Preliminary dialysis of the serum or electrophoretic fractions with the starting buffer produced some precipitation of euglobulins. There was, however, no demonstrable loss of antibody activity in the supernatant, and the redissolved precipitate showed negligible activity.

Concentration of Antibodies

In preparation for further physicochemical study, certain electrophoretic or chromatographic antibody-containing fractions were pooled and concentrated by pervaporation on a revolving wheel before an electric fan at room temperature. Evaporation was sufficiently rapid to maintain the temperature of the solution at approximately 15°C.

Mercaptan Treatment

Dissociation of 19S γ-globulins was effected by treating convenient dilutions of each pathologic serum with 0.1M 2-mercaptoethanol or with 0.1M DL-penicillamine, using modifications of previous methods. After 48 hours at room temperature, the mercaptoethanol was removed by thorough dialysis against TBS, prior to serologic testing. Penicillamine treatment was carried out at 37°C for 24 hours. The penicillamine was not removed prior to serologic testing since it was found to have no effect on the serologic reactions when added to untreated sera immediately before testing.

RESULTS

Physicochemical Studies

Electrophoresis. Sera J. F. and N. P. (table 1) were subjected to starch block electrophoresis and the eluted fractions were titrated in the presence of fresh serum for complete and incomplete cold antibody activity (fig. 1). Cold antibody was demonstrable over a broad range in the γ- and β-globulin regions. There was no demonstrable separation of the incomplete from complete antibody activity, both having essentially identical ranges and peaks.

Ultracentrifugation. Sera N. P., J. F. and W. B. were each subjected to density gradient preparative ultracentrifugation on several occasions. Since previous work had established the 7S nature of incomplete anti-Rh antibody and the 19S nature of ("complete") pathologic cold agglutinins, advantage of this knowledge was taken in localizing the 7S and 19S globulin regions after ultracentrifugation (see Methods). The direct cold agglutinin activity was found only in the lower region, anti-Rh activity in the middle region, and albumin, marked by Evans blue dye, at a somewhat higher level (table 2). Of particular interest was the finding that the incomplete cold antibody activity was found not with the incomplete anti-Rh antibody, but in precisely the same fractions as the direct cold agglutinins, indicating that both cold antibody functions are associated with high molecular weight globulins. As expected, the incomplete cold antibody activity was demonstrable only when fresh normal serum had been added to the incubation.

Chromatography. Anion exchange fractionation on a DEAE-cellulose column was employed in a further attempt to separate the complete and incomplete cold antibody activities of sera N. P. and J. F. Previous chromatographic studies of normal human γ-globulin had shown that the majority of 7S γ-globulins
ZONE ELETROPHORESIS
N.P. Serum

Fig. 1.—The titers of "complete" and "incomplete" pathologic cold antibody activities (vertical bars) in the fractions obtained by starch block electrophoresis of N.P. serum. The protein concentration of each fraction (linear curve) was determined by the Folin-Ciocalteu reaction (U.S. Army Photograph).

are eluted in the first protein peak and that the last fractions eluted contain the 19S γ-globulins with a small quantity of 7S material.

The cold antibodies of N. P. were applied to the column either as whole serum or as the concentrated antibody-containing electrophoretic fractions (fig. 2). In both instances, cold antibody activity was found only in the late chromatographic fractions. There was no demonstrable dissociation of complete and incomplete cold antibody activities, the titers of each remaining generally proportional to their relative titers in the unfractionated serum. Comparable results were obtained with serum J. F. In all chromatographic studies, the high phosphate concentration in the last 15–20 tubes inhibited the incomplete antibody activity, probably through C' inhibition, without disturbing direct cold agglutination. Dialysis against 0.15M saline prior to incubation readily restored the incomplete cold antibody activity of these fractions. Dialysis of earlier fractions did not alter their antibody activity.

Mercaptan treatment. Recent investigations have demonstrated that mercaptan compounds are capable of dissociating 19S human γ-globulins into smaller (6.6–7S) units and that 19S antibodies, including "complete" cold agglutinins, so treated lose their biological activity. Human 7S antibodies are not affected. By analogy to certain incomplete isoantibodies, incomplete cold antibody might be presumed to be a 7S γ-globulin, although
Table 2.—Density Gradient Ultracentrifugation of Pathologic Cold Antibodies Relative to Added Anti-Rh Antibodies and to Albumin

<table>
<thead>
<tr>
<th>Ultracentrifugal Fraction*</th>
<th>Albumin Anti-Rh Titer†</th>
<th>Incomplete Anti-Rh Titer†</th>
<th>Cold Antibody Titer‡</th>
<th>Complete§</th>
<th>Incomplete¶</th>
</tr>
</thead>
<tbody>
<tr>
<td>Upper 1</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>2</td>
<td>2+</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>3</td>
<td>3+</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>4</td>
<td>trace</td>
<td>1:8</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>5</td>
<td>—</td>
<td>1:4</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>6</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>7</td>
<td>—</td>
<td>—</td>
<td>1:32</td>
<td>1:8</td>
<td>1:8</td>
</tr>
<tr>
<td>8</td>
<td>—</td>
<td>—</td>
<td>1:32</td>
<td>1:10</td>
<td>1:10</td>
</tr>
<tr>
<td>Lower 9</td>
<td>—</td>
<td>—</td>
<td>1:8</td>
<td>1:8</td>
<td>1:8</td>
</tr>
</tbody>
</table>

*Serum J. F., 1:2 initial dilution, with added Evans blue dye and incomplete anti-Rh antibodies; 35,000 rpm for 14 hours.
†Determined by agglutination of O Rh positive red cells by “anti-γ globulin” serum after 37 C. incubation with each fraction.
‡Determined after 2 C. incubation of O Rh neg. red cells with each fraction in presence of added complement.
§“Complete” activity = direct cold agglutination; “incomplete” activity = antiglobulin reaction, using an “anti-non-γ-globulin” serum.

The foregoing ultracentrifugal studies contradict such an assumption. It was of interest, therefore, to determine whether the incomplete cold antibody activity of the sera under study would be affected by mercaptan treatment.

The results of serum treatment with 0.1M 2-mercaptoethanol and with 0.1M DL-penicillamine are summarized in table 3. In two sera, all evidence of both complete and incomplete antibody was destroyed. The most potent serum (N. P.) retained moderate activity after mercaptoethanol treatment but showed a significant and proportionate fall in the titer of both forms of antibody activity; all activity was destroyed by exposure to a slightly higher concentration (0.13M) of penicillamine. Before titration, each treated and control serum was carefully tested with sensitized sheep red cells for anticomplementary activity; none was observed. Thus, “incomplete” and “complete” cold antibody activities are equally affected by mercaptan treatment. Moreover, a known 7S γ-globulin, incomplete anti-Rh, antibody, was unaffected by the same treatment carried out simultaneously (table 3). These results are in keeping with the ultracentrifugal data and indicate that both forms of pathologic cold antibody activity reside in 19S molecules.

Serologic Studies

The four sera under investigation (table 1) conformed in their general serologic behavior to the pattern described in the opening remarks of this paper. The sera were serologically inert at 37 C. and had only slight activity at room temperature. Hemolysis was not encountered at the physiologic pH employed in all serologic reactions.

Attempts at preferential absorption. In a further effort to determine whether the complete and incomplete cold antibody activities of the pathologic sera resided in distinct antibody molecules, several attempts were made to absorb
preferentially from a given serum a relatively greater proportion of one antibody activity than the other. For this purpose a special protocol, schematically outlined in figure 3, was used. In this protocol, following cold incubation of a pathologic serum with a large number of normal red cells and with C', the tube was centrifuged at 4 C. and a "4° supernatant" removed before warming was allowed. The cells were washed at 4 C. This washing was necessarily imperfect due to persisting agglutination at this temperature. After the final 4 C. wash the packed cells were warmed to 37 C. in the presence of a volume of warmed triethanolamine buffered saline (TBS) equal to the volume of the serum dilution employed in the original incubation. A "37° supernatant" was removed.* Then, by titering the 4 C. and 37 C. supernatants, attention was directed to: (a) antibody which did not attach to the cells in the cold and therefore remained in the "4° supernatant"; and (b) antibody which apparently had attached to the cells at 4 C. but was removed by warming in the "37° supernatant". Either the "4° supernatant" or the "37° supernatant" could then be subjected to another cycle of absorption.

To be detectable, incomplete cold antibody activity requires the presence of C'; complete cold antibody activity does not. This difference might appear to afford a simple means of serologic separation of the two activities. Preliminary studies, however, clearly showed that both complete and incomplete antibody activities of a pathologic serum were absorbed by normal erythro-

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*This is really an eluate but the term was avoided to prevent confusion with the serologically inert "eluates" obtained by other procedures from washed, "non-γ-globulin" coated red cells.14,17
Table 3.—In Vitro Effect of Mercaptans on Pathologic Cold Antibodies

<table>
<thead>
<tr>
<th>Serum</th>
<th>Mercaptoethanol Effect on Titors of Antibody Activity*</th>
<th>Penicillamine Effect on Titors of Antibody Activity*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Complete† Incomplete†</td>
<td>Complete† Incomplete†</td>
</tr>
<tr>
<td>W. B. control†</td>
<td>1:80 1:40</td>
<td>1:160 1:40</td>
</tr>
<tr>
<td>W. B. treated‡</td>
<td>neg. neg.</td>
<td>neg. neg.</td>
</tr>
<tr>
<td>J. F. control</td>
<td>1:320 1:160</td>
<td>1:640 1:160</td>
</tr>
<tr>
<td>J. F. treated</td>
<td>neg. neg.</td>
<td>neg. neg.</td>
</tr>
<tr>
<td>N. P. control</td>
<td>1:10,240 1:2560</td>
<td>1:15,360 1:7680</td>
</tr>
<tr>
<td>N. P. treated</td>
<td>1:160 1:80</td>
<td>neg. neg.</td>
</tr>
<tr>
<td>Anti-Rh control</td>
<td>neg. 1:80</td>
<td>neg. 1:80</td>
</tr>
<tr>
<td>Anti-Rh treated</td>
<td>neg. 1:80</td>
<td>neg. 1:80</td>
</tr>
</tbody>
</table>

*The titers shown are with respect to the neat sera, although neat sera were not used in the experiment (see Methods). The final dilutions of the sera during treatment were as follows: W. B. 1:10, J. F. 1:20, N. P. 1:40 and 1:30, and anti-Rh 1:10.

†Complete activity = direct cold agglutination; incomplete activity = antiglobulin reaction.

‡Controls consisted of aliquots of the same sera handled identically except that an equal volume of buffered saline alone was added in lieu of the mercaptan.

§Treatment with 0.1M concentration of the mercaptan in buffered saline. The only exception was the use of 0.13M penicillamine with serum N. P.

cytes and subsequently recovered in the "37° supernatant" (fig. 3), whether or not C' had been present in the original incubation. The presence of incomplete cold antibody activity in the "37° supernatant" was demonstrated by incubation with fresh red cells and C', after which the cells were found to react positively in the antiglobulin test. That this incomplete antibody had not simply been passively entrained in the interstices of the direct cold agglutinates and subsequently released into the "37° supernatant" by warming was shown by producing the same result using a sufficient quantity of absorbing red cells to leave the "4° supernatant" devoid of demonstrable antibody. Obviously, the quantity of entrained antibody cannot exceed the antibody content of the supernatant fluid, i.e. the "4° supernatant", in which the possibly entraining agglutinates were formed. It can be concluded that while C' is essential for the production of a stable erythrocyte coating, it is not required for the active, thermally reversible attachment of the incomplete cold antibodies themselves, i.e., antibodies possessing the serologic activity attributed to "incomplete" cold antibodies. This conclusion has also been reached by Dacie.²

Further attempts at preferential absorption involved multiple cycles of absorption, according to the outline in figure 3, in an effort to reveal any differences of antibody affinity that might indicate two independent antibodies. In one set of experiments, the four pathologic sera were subjected to three or four cycles of absorption in the presence of C'. Each absorption after the first was performed upon the "4° supernatant" from the previous cycle, after a small aliquot had been removed for subsequent titration. When the cycles of absorption were completed, the titers of both the 4 C. and 37 C. supernatants from each cycle were determined. As a reference for the relative titers of complete and incomplete antibody activities before absorption, an aliquot of the unab-
Dilution of Serum containing cold antibodies + Normal red cells + Complement

\[
\text{Incubation at } 2^\circ\text{C} \\
4^\circ\text{centrifugation}
\]

4^\circ\text{Supernatant} \quad \text{Agglutinated Cells}

3 washes with 4^\circ\text{ saline}, gentle stirring, 4^\circ\text{ centrifugations}

Washed, agglutinated, packed cells

37^\circ\text{ bath, } 10 \text{ min., } 37^\circ\text{TBS added^*}, gentle stirring

Suspension of unagglutinated cells

brief centrifugation at room temperature

37^\circ\text{Supernatant} \quad \text{Unagglutinated Cells (with 'non-\text{y-globulin}' coating)}

Fig. 3.—Schematic presentation of the serologic protocol employed in studies of preferential absorption (U. S. Army Photograph).

^*Triethanolamine buffered saline, volume equal to the volume of the fluid containing the cold antibodies in the first step of this schema.

sorbed pathologic serum was simultaneously titered. Identical preparations of red cells, C', and antiglobulin serum were used in all of these titrations. The results for J. F. serum (table 4) are representative. Stepwise reduction in the total antibody remaining in the "4^\circ" supernatants" did not demonstrably alter the relative proportions of complete and incomplete antibody activity.

Another approach to preferential absorption was based upon the following reasoning. If the "non-\text{y-globulin}" erythrocyte coating, which is not detectably reversed by warming, included incomplete antibody irreversibly bound to the cell through a "cementing" action^3 of C' with the relative exclusion of complete antibody, then a cumulative depletion of incomplete antibody might be expected to occur during repeated absorptions in which large numbers of red cells acquired a "non-\text{y-globulin}" coating while the complete agglutinins were repeatedly eluted by warming. This approach raises the question of differing degrees of reversibility of complete versus incomplete antibody attachment in the presence of C'. Thus, any serologically detectable relative depletion of incomplete antibody activity that might occur would be expected to appear in a "37^\circ\text{ supernatant}" (fig. 3) since the latter contains whatever thermally revers-
ibly bound antibodies, both complete and incomplete, can be recovered from the absorbing red cells.

Accordingly, two parallel absorption sequences were carried out as outlined in figure 3, each absorption after the first being performed upon the “37° supernatant” from the previous cycle. In one sequence, an excess of C′ was provided at the beginning of each cycle; the other sequence lacked C′ throughout. In all other respects the handling of the two sequences was the same. The red cells from each absorption performed in the presence of C′ showed a “non-\(\gamma\)-globulin” coating; those of the sequence lacking C′ remained negative in all antiglobulin tests. The results of this experiment on serum N. P. (table 5) are representative. After three cycles of absorption, there was no disproportionate reduction in the incomplete antibody activity of the “37° supernatant” in the C′-containing sequence as compared to the C′-free sequence. In this experiment, the proportions of the reacting cold antibodies and red cells were adjusted to allow complete absorption of the antibodies in each cycle. All of the “4° supernatants” of both sequences therefore lacked demonstrable antibody. This arrangement not only minimized the theoretical danger of entrainment but, by providing for absorption of all detectable antibody in one step, also avoided the possible selective absorption of antibodies with varying serologic properties such as avidity. In other experiments, somewhat smaller proportions of red cells were used for as many as four cycles of absorption with similar results.

Reactivity with “Bombay” and I-negative erythrocytes. In recent years, evidence has been presented that pathologic cold agglutinins have a specificity for a nearly universal antigenic determinant of human red cells designated as “I”\(^{36,39}\) and that the incomplete cold antibodies present in most normal sera have anti-H specificity.\(^{40}\) Since the rare “Bombay” and I-negative (“i”) types of human red cells lack demonstrable H antigen\(^{11,12}\) and “I” antigen\(^{36,39}\) respectively, a study was undertaken to determine whether or not the complete and incomplete antibody activities of the pathologic sera could be separated on the basis of their reactivities with these cells. As a matter of interest, pathologic and “normal” cold antibodies were also compared in this respect.

The reactivities of the four pathologic sera with “Bombay” red cells (table 6) were entirely comparable to their reactivities with normal group O erythrocytes. Thus, neither the incomplete nor the complete pathologic cold antibody activity exhibits anti-H specificity. In contrast, three of the pathologic sera were completely unreactive with I-negative red cells (table 6). Serum J. F., however, retained moderate activity of both complete and incomplete types against I-negative cells, though the titers were greatly reduced. Absorption of this serum with a large volume of I-negative cells completely removed its reactivity with I-negative cells without affecting its reactivity with normal red cells (table 6), suggesting that the cold antibodies of this serum are not entirely of “anti-I” specificity. In any event, the complete and incomplete cold antibody activities of the four pathologic sera could not be separated on the basis of differing specificity for “Bombay” or I-negative erythrocytes.

When tested with several normal group O erythrocytes, each of the normal
Table 4.—*Effect of Repeated Absorptions on the Relative Titers of Complete and Incomplete Cold Antibody Activities*

<table>
<thead>
<tr>
<th>Absorption Step</th>
<th>Material Absorbed</th>
<th>Material Titered</th>
<th>10</th>
<th>20</th>
<th>40</th>
<th>80</th>
<th>160</th>
<th>320</th>
<th>640</th>
<th>1280</th>
<th>2560</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1:10 J. F. Serum</td>
<td>Unabsorbed</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>2</td>
<td>trace</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1:10 J. F. Serum</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>4 C. supernatant, step 1½</td>
<td>Unabsorbed</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>3</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1:10 J. F. Serum</td>
<td>4</td>
<td>3</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>37 C. supernatant, step 1½</td>
<td>Unabsorbed</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>3</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1:10 J. F. Serum</td>
<td>4</td>
<td>4</td>
<td>3</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>4 C. supernatant from step 1</td>
<td>Unabsorbed</td>
<td>4</td>
<td>4</td>
<td>3</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1:10 J. F. Serum</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>trace</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>37 C. supernatant, step 2</td>
<td>Unabsorbed</td>
<td>4</td>
<td>4</td>
<td>3</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1:10 J. F. Serum</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>trace</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>4 C. supernatant from step 2</td>
<td>Unabsorbed</td>
<td>4</td>
<td>3</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1:10 J. F. Serum</td>
<td>3</td>
<td>1</td>
<td>trace</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td></td>
<td>37 C. supernatant, step 3</td>
<td>Unabsorbed</td>
<td>4</td>
<td>2</td>
<td>trace</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>1:10 J. F. Serum</td>
<td>3</td>
<td>2</td>
<td>trace</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>4 C. supernatant from step 3</td>
<td>Unabsorbed</td>
<td>4</td>
<td>3</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1:10 J. F. Serum</td>
<td>trace</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

*As a reference for the relative amounts of "complete" and "incomplete" pathologic cold antibody activities, an aliquot of unabsorbed 1:10 J. F. serum was titered at each step. The results are presented only once since the four titrations did not differ significantly. A single preparation of 1:10 serum was the source of both the aliquot subjected to absorption and the aliquot serving as reference.

†The numbers indicate the strength of the individual reactions, i.e., 4+, 3+, etc., as explained in Methods. In each row, direct cold agglutination (CAPS) is written above, with antoglobulin reaction (*italics*) below.

‡The titers of both 4 C. and 37 C. supernatants are based upon calculated true dilutions with respect to the original undiluted serum. The readdition of C before each absorption necessitated a constant increment of dilution. In the second and fourth steps, the small volume removed for titration was further diluted to fit the titration pattern, e.g., 1:20 and 1:40.
Table 5.—Relative Titers of Complete and Incomplete Cold Antibody Activities After Three Absorptions in the Presence or Absence of Complement

<table>
<thead>
<tr>
<th>Source of Antibody</th>
<th>Reciprocals of Titers with Graded Reactions</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>160</td>
</tr>
<tr>
<td>Aliquot of unabsorbed</td>
<td>4*</td>
</tr>
<tr>
<td>N. P. serum</td>
<td>2</td>
</tr>
<tr>
<td>Third 37 C. supernatant, C'-containing sequence</td>
<td>4</td>
</tr>
<tr>
<td>Third 37 C. supernatant, C'-free sequence</td>
<td>2</td>
</tr>
</tbody>
</table>

*In each row, direct agglutination ("complete" activity) is written above (CAPS), with antiglobulin reaction ("incomplete" activity) below (italics).

Table 6.—Reactivity of Pathologic Cold Antibodies with Normal, "Bombay," and "I-Negative" Red Cells

<table>
<thead>
<tr>
<th>Pathologic Serum</th>
<th>vs. Normal red cells</th>
<th>vs. Bombay red cells</th>
<th>vs. I-neg. red cells</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Complete*</td>
<td>Incomplete*</td>
<td>Complete</td>
</tr>
<tr>
<td>W. B.</td>
<td>640</td>
<td>160</td>
<td>640</td>
</tr>
<tr>
<td>W. N.</td>
<td>640</td>
<td>40</td>
<td>640</td>
</tr>
<tr>
<td>N. P.</td>
<td>10,240</td>
<td>5120</td>
<td>10,240</td>
</tr>
<tr>
<td>J. F.</td>
<td>1280</td>
<td>640</td>
<td>1280</td>
</tr>
<tr>
<td>J. F. absorbed†</td>
<td>1280</td>
<td>320</td>
<td>nag.</td>
</tr>
</tbody>
</table>

*Complete = direct cold agglutination; incomplete = antiglobulin reactivity.
†Prior absorption with excess of washed "I-negative" erythrocytes.

Sera exhibited incomplete cold antibody activity in low titer, also of the "non-\(\gamma\)-globulin" type (table 7). This activity was uniformly absent when these sera were reacted with "Bombay" red cells (table 7) in contrast to the behavior of the pathologic sera (table 6).

Some normal sera also exhibited direct cold agglutination of normal group O erythrocytes, although only 2 (J. C., J. L.) gave significant titers (table 7). Serum J. C. agglutinated directly both normal and "Bombay" cells. Absorption of this serum with washed "Bombay" cells completely removed the capacity of the serum to agglutinate normal cells directly without affecting the incomplete antibody activity against these normal cells (table 7). Thus, in this serum, while the normal incomplete cold antibodies may have had anti-H specificity, the normal complete cold antibodies probably did not. (J. C., a male, gave no history of previous viral pneumonia, infectious mononucleosis, transfusion or hematologic disorder). In contrast, absorption of J. L. serum with "Bombay" cells affected neither complete nor incomplete antibodies (table 7), suggesting that in this normal serum both antibody activities were of anti-H specificity.

Further Observations

No pathologic serum possessing a high titer of incomplete cold antibody ("anti-I") alone has been encountered in this laboratory. One pathologic serum, not among those reported in the studies of this paper, on first examination was found to have a 1:80 titer of direct cold agglutination but no incomplete ac-
Table 7.—Reactivity of Normal Cold Antibodies with "Bombay" and Normal Red Cells

<table>
<thead>
<tr>
<th>Normal Serum</th>
<th>vs. Bombay Red Cells</th>
<th>vs. Normal Red Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Complete</td>
<td>Incomplete</td>
</tr>
<tr>
<td>N. T.</td>
<td>neg.</td>
<td>neg.</td>
</tr>
<tr>
<td>E. M.</td>
<td>neg.</td>
<td>neg.</td>
</tr>
<tr>
<td>M. M.</td>
<td>neg.</td>
<td>neg.</td>
</tr>
<tr>
<td>C. R.</td>
<td>neg.</td>
<td>neg.</td>
</tr>
<tr>
<td>C. B.</td>
<td>neg.</td>
<td>neg.</td>
</tr>
<tr>
<td>J. L.</td>
<td>neg.</td>
<td>neg.</td>
</tr>
<tr>
<td>J. C.</td>
<td>4</td>
<td>neg.</td>
</tr>
<tr>
<td>J. C. absorbed†</td>
<td>neg.</td>
<td>8</td>
</tr>
<tr>
<td>J. L. absorbed†</td>
<td>8</td>
<td>4</td>
</tr>
</tbody>
</table>

*Complete = direct cold agglutination; incomplete = antiglobulin reactivity.
†Prior absorption with excess of washed "Bombay" erythrocytes.

tivity, suggesting that in this instance the complete cold agglutinin may have existed separately. This serum, however, was found to be anticomplementary. A "37° supernatant" (fig. 3) was prepared from red cells incubated with this serum in the absence of added C'. Subsequent titration of this "37° supernatant" revealed both complete and incomplete activities. It seems likely that isolation of the cold agglutinins by elution freed them of the anticomplementary effect of the whole serum. Similar observations were made on serum W. B. when used at a concentration exceeding 1:10 and on certain electrophoretic fractions of serum J. F. Thus, sera exhibiting direct cold agglutination should not be declared free of incomplete cold antibody activity unless such factors have been ruled out.

**DISCUSSION**

Attempts to separate the complete and incomplete pathologic cold antibody activities of four sera by both physicochemical and serologic techinics were completely unsuccessful. Both activities were found to have the same electrophoretic mobilities, ultracentrifugal characteristics, chromatographic behavior, and susceptibility to mercaptan treatment. Within the limitations of the serologic methods, the two antibody activities exhibited equal affinity for a given sample of red cells under varying circumstances, and complete correspondence in the tests of serologic specificity employed.

The simplest interpretation of these results is that both the complete and the incomplete serologic activities of pathologic cold antibodies can be ascribed to a single antibody, the pathologic cold agglutinin. According to this concept, the complete activity of the antibody is evidenced by direct agglutination of red cells in the cold, which is reversible simply by warming. This antigen-antibody complex, in turn, causes binding of non-γ-globulin proteins from fresh serum, presumably complement components, resulting in the incomplete activity of the antibody, i.e., a positive antiglobulin reaction. This secondary fixation of protein or complement (C') to the cell surface is not detectably reversible. The postulate of a separate incomplete cold antibody is unnecessary to account for the observations.
COMPLETE AND INCOMPLETE COLD AGGLUTININS

Failure of separation of the two biological effects does not, of course, rigidly prove the identity of the agent(s) responsible for these effects. The constant ratio of complete to incomplete antibody activity under a relatively wide variety of experimental circumstances is highly suggestive. Unfortunately, however, the method of titration used is insufficiently precise to permit the conclusion that this ratio was in fact absolutely constant. Yet, if there are two antibodies involved, they have a most remarkable similarity in all respects studied, and the similarities in physicochemical properties stand in contrast to the differences so well defined for the separate complete and incomplete antibodies in the Rh system.7,10

If this unitary concept is correct, it follows, as Dacie and associates have also pointed out,11 that a positive antiglobulin test resulting from an apparently classical immunologic reaction does not necessarily imply activity of a distinct incomplete antibody. This corollary appears to have applicability to other hemagglutinating systems, e.g., certain human blood group antibodies.

In the experiments attempting preferential depletion of incomplete activity in the presence of C' (tables 4 and 5), the recovery in the “37° supernatants” (cf. fig. 3) of incomplete activity in quantities comparable to the recovered complete activity is entirely consonant with the concept that both activities reside in a single antibody, the great majority of which is reversibly attached to the red cells. Moreover, the data in table 5, showing no detectable decrease in either type of activity in the C'-containing sequence compared to the C'-free sequence, suggest that if antibody is irreversibly bound to the cells in the process of C'-fixation, it is too small a quantity to be serologically detected. While the relative insensitivity of hemagglutination as a measurement of antibody must be recognized, it is conceivable that previous failures14,17 to elute detectable quantities of antibody from washed “non-γ-globulin”-coated red cells may reflect, in part, the presence of a relatively small quantity of antibody in this coating as well as probable firm bonding between the red cell and the antibody-complement coating material. It is theoretically possible, but perhaps unlikely, that no antibody remains in the “non-γ-globulin” coating, i.e., that the C' coating may persist without the presence of antibody once fixation of C' has been achieved.

Using penicillamine (β,β-dimethylcysteine) in patients with Waldenström’s macroglobulinemia, Bloch and associates43 have recently demonstrated the ability of mercaptan compounds to bring about in vivo destruction of 19S γ-globulins. On the assumption that the cold agglutinins mediate the accelerated red cell destruction in the “cold type” of acquired hemolytic anemia, the findings that both the direct agglutinating and the possibly more clinically important44 C'-fixing activities of these pathologic sera reside in mercaptan-sensitive 19S γ-globulin (tables 2 and 3) give theoretical impetus to the investigation of therapeutic use of mercaptan compounds in this disorder.48

The reported anti-I specificity of pathologic cold antibodies36,39 was apparently borne out in three of the sera studied, but not entirely in a fourth serum obtained from a patient in whom the clinical picture was in no way distinctive and in a typical clinical context for the presence of pathologic cold antibodies. This suggests possible greater complexity in the serologic specificity
of pathologic cold antibodies than was evident from earlier studies with I-negative ("i") red cells. On the other hand, those antibodies reactive with I-negative cells may be a normal constituent of this serum, unrelated to the stimulus of viral pneumonia. Regrettably, this serum was exhausted before the cold antibodies lacking anti-I specificity could be studied for other specificities such as anti-H or anti-P.

The failure of normal incomplete cold antibody to react with "Bombay" erythrocytes, which lack detectable H antigen,41,42 confirms the anti-H specificity of these antibodies. Crawford and associates had originally reached the same conclusion by another approach.46 The specificity of normal complete cold agglutinins46 requires further study. Jenkins, Tippett and their associates37,38 found that the complete cold agglutinins of some normal sera seemed to have anti-I specificity. On the other hand, the reactions of other normal complete cold agglutinins (table 7) suggest possible anti-H specificity like that of normal incomplete cold antibodies. The "Bombay" cells used were certainly not deficient in "I" antigen (table 6). In any event, these studies of specificity indicate that pathologic cold agglutinins are serologically unrelated to the incomplete cold antibodies of normal sera. They may be related to the complete cold agglutinins in some normal sera but not in others.

The occurrence in some normal sera of incomplete cold antibody with no demonstrable complete antibody, or with a significantly lower titer of complete antibody (table 7), stands in contrast to the observations on the pathologic sera in which incomplete activity was not found in the absence of or in excess of the complete activity by the methods and reagents used. The question of whether or not the complete and incomplete cold antibodies of normal sera are distinct entities awaits, in part, clarification of the specificity of the complete antibodies. In serum J. C. (table 7) they appear to be distinct; in serum J. L. they may not be. In sera M. M. and C. B. the incomplete antibody may be an independent entity. A normal serum with a sufficiently high antibody titer to approach this question by physicochemical means was not available. In an earlier study,27 however, the normal "complete" cold agglutinins of several sera were shown to be in the high molecular weight class.

The significance of pathologic cold agglutinins and the mechanism(s) responsible for their appearance in a variety of clinical circumstances pose challenging, unanswered questions. The recent discovery that these "non-specific" agglutinins apparently do have specificity for an antigen which is almost universally present on human red cells enhances their immunologic status and interest. In the light of current immunologic concepts,47,48 the appearance in otherwise healthy persons with viral pneumonia of high titers of agglutinins reactive with the "I" antigen of their own red cells, occasionally with hemolytic consequences, is as remarkable as would be a comparable production of anti-A agglutinins in persons of blood group A. In the course of this study, the apparently similar specificity, i.e. anti-I, of the cold agglutinins arising in such seemingly disparate conditions as primary atypical pneumonia (e.g. patient W. B.) and idiopathic "autoimmune" hemolytic anemia (patient N. P.) was noted with interest. This similarity may be accidental. On the other
hand, pathologic cold agglutinins may be conceived to be autoantibodies and one might speculate upon a possible common pathogenetic pathway in these two diseases leading to essentially the same immunologic alteration, with the pathway affected transiently in viral pneumonia and more chronically in hemolytic anemia.

**SUMMARY**

Several human pathologic sera containing high titered cold agglutinins were studied to determine whether the serologic activity ascribed to an "incomplete cold antibody" could be separated from the "complete" cold agglutinin activity. Separation was not achieved by physicochemical methods, including zone electrophoresis, density gradient ultracentrifugation, and anion exchange chromatography. Both activities were susceptible to destruction by mercaptans. Neither activity could be differentially absorbed from the sera. Using "Bombay" and I-negative ("i") red cells, a difference in specificity of the two activities for the H or I antigen of human erythrocytes could not be demonstrated. The simplest interpretation of these findings is that there is only one antibody involved, the cold agglutinin, and that the serologic manifestation usually attributed to an additional "incomplete cold antibody", i.e. the production of a positive antiglobulin reaction of the "non-γ-globulin" type, results from an interaction of complement components with the cold agglutinin-erythrocyte complex.

Three of these cold agglutinating sera were unreactive with I-negative erythrocytes, in keeping with the reported anti-I specificity of these antibodies. A fourth serum retained moderate, though greatly reduced, activity against these cells, and the interpretation of this finding is discussed.

The anti-H specificity of the incomplete cold antibodies in normal human sera was confirmed by their failure to sensitize "Bombay" erythrocytes. This was in sharp contrast to the excellent reactivity of the pathologic sera with these cells, demonstrating that pathologic cold agglutinins are unrelated to the incomplete cold antibodies present in most normal sera.

**SUMMARIO IN INTERLINGUA**

Plure pathologic seros human con alte titros de cryoagglutinina esseva studiate pro determinar si le activitate serologic ascribite a un "incomplete cryoanticorpore" pote esser separate ab le activitate de "complete" agglutinina. Il non esseva possibile separar le duo per methodos physicochimic, incluse electrophorese de zonas, ultracentrifugation a gradiente de densitate, e chromatographia a excambio anionic. Ambe activitates esseva susceptible de esser destruite per mercaptanos. Ni le un ni le altere poteva esser absorbite differentialmente ab le seros. Nulle specificitate del duo activitates pro le antigeno H o le antigeno I de erythrocytos human poteva esser demonstrate con le uso de erythrocytos "Bombay" e I-negative ("i"). Le interpretation le plus simple de iste constatationes es que solmente un anticorpore es interessate in iste situationes, i.e. le cryoagglutinina, e que le manifestation serologic que es usualmente attribuite al existentia additional de un incomplete cryo-
anticorpore—i.e. le production de un positive reaction antiglobulinic del typo "non globulina gamma"—resulta ab un interaction de componentes de complemento con le complexo de cryoagglutinina e erythrocytos.

Tres de iste cryoagglutinante seros esseva non-reactive con erythrocytos I-negative, de accordo con le reportate specificitate anti-I de iste anticorpores. Un quarte sero reteneva moderate, ben que grandemente reducite grado de activitate contra iste cellulas. Le interpretation de iste constatationes es discutite.

Le specificitate anti-H de incomplete cryoanticorpore in normal sero human esseva confirmate per le constatation que illo non effectua un sensibilisation de erythrocytos "Bombay." Isto contrastava marcatemente con le excellent reactivitate del seros pathologic con ille cellulas, de manera que on pote reguardar como demonstrate le facto que cryoagglutininhas pathologic non es relacionate al incomplete cryoanticorpore que es presente in le majoritate del seros normal.

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COMPLETE AND INCOMPLETE COLD AGGLUTININS


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Scott N. Swisher, M.D., Associate Professor of Medicine; Head, Hematology Unit, Department of Medicine, University of Rochester School of Medicine and Dentistry, Rochester, N. Y.
The Unitary Nature of "Complete" and "Incomplete" Pathologic Cold Hemagglutinins

JOHN P. LEDDY, NORMA C. TRABOLD, JOHN H. VAUGHAN and SCOTT N. SWISHER