THE IN VIVO METABOLISM OF RADIOIODINATED COLD AGGLUTININS OF ANTI-I SPECIFICITY

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SUMMARY

1. The metabolism of six purified and radioiodinated cold agglutinins has been studied in five patients with chronic cold haemagglutinin disease and in six normal controls. Two of the patients showed overt haemolysis at the time of this study and all had complement components adsorbed to the surface of their red cells.

2. The mean fractional catabolic rate (FCR) of the cold agglutinins, studied in the patients from whom they were isolated (14.5%/day), was similar to the mean FCR of the same cold agglutinins in normal controls (15.6%/day) and there was no correlation between the FCR and the presence of haemolysis. Exposure to cold with a concomitant increase in haemolysis during the course of one of these studies had no effect on the catabolic rate of the cold agglutinin.

3. Individual values for the FCR ranged from 10.8 to 19.4%/day (corresponding to half-lives of 4.9-7.8 days) and appeared to be characteristic for each recipient. When two different cold agglutinins were studied simultaneously in the same subject their catabolism was virtually identical.

4. Untreated patients with chronic cold haemagglutinin disease were calculated to be synthesizing IgM at approximately ten times the normal rate. The fall in serum IgM concentration and agglutinin and haemolysin titres, following treatment with alkylating agents, appeared to be due to a diminution in the rate of synthesis and not due to increased catabolism.

5. '7S' subunits of one of the cold agglutinins, which retained cold haemagglutinating activity, were rapidly catabolized in a normal subject.

The cold haemagglutinin syndrome is characterized by a chronic haemolytic anaemia of many years duration, typically of mild to moderate intensity which becomes worse in cold weather (Schubothe, 1952; Dacie, 1962). Haemoglobininaemia, haemoglobinuria, jaundice and Ray-
nau̇d's phenomena may occur on exposure to cold. The cold autoantibodies, usually specific for the I-antigen (Wiener et al., 1956; Van Loghem et al., 1962; Harboe & Deverill, 1964), are strongly agglutinating with titres commonly within the range 2000–128 000 at 4°C and a thermal amplitude stretching up to 28–32°C. They are complement-fixing and lytic to varying degrees, and may be present in such high concentrations as to be visible as distinct bands on electrophoresis, when they migrate in the β to γ position (Christenson & Dacie, 1957).

A number of studies have shown that these antibodies are IgM immunoglobulins (Franklin & Fudenberg, 1964) with almost exclusively type Kappa light chains (Harboe et al., 1965; Cooper & Worlledge, 1967). Electrophoretic studies and N-terminal amino-acid analyses of separated light chains have provided evidence (Cooper, 1968; Cohen & Cooper, 1968) that the parent antibodies are monoclonal.

By virtue of their high serum concentration and monoclonal nature the antibodies strikingly resemble the M-proteins of multiple myeloma and Waldenström's macroglobulinaemia. Clinically, however, chronic cold haemagglutinin disease may be hardly progressive during years of observation and there may be no perceptible change in the serum IgM concentration over months or years (Cooper, A.G. & Hobbs, J.R., unpublished).

Little is known about the synthesis, metabolism and distribution of the cold antibodies or whether repeated adsorption to and elution from red cells, and their participation in complement-fixation, alters their molecular structure, so affecting their catabolism.

The present report describes metabolic studies on five patients with chronic cold haemagglutinin disease, two of whom were suffering from overt haemolysis, and on six normal controls using serologically active ¹²⁵I and ¹³¹I-labelled cold agglutinins.

**MATERIALS AND METHODS**

*Patients with chronic cold haemagglutinin disease*

Three male and two female patients volunteered for these studies. Their ages ranged from 52 to 84 years. The metabolic studies were performed at times when the patients were admitted to the Hammersmith Hospital for reassessment of their disease or for control of immunosuppressive therapy. The aims and the possible hazards of the studies were explained to the patients before permission to carry out the investigations was requested. Their clinical and laboratory data are summarized in Table 1. The cold agglutinin of a sixth patient (A.C.) was studied in a normal control only. All the patients presented clear clinical evidence of the disease for several years and had experienced Raynaud's phenomena and episodes of anaemia, haemoglobinuria and jaundice during cold weather on several past occasions. At the time of this study, W.S. and E.B. were suffering from overt haemolysis. Renal and cardiac function were normal and no patient had oedema or proteinuria. In each case the direct antiglobulin test was strongly positive and of the complement-only type. Agglutinin titres at 4°C and lysin titres at 20°C were stable in four patients, three of whom were on long-term treatment with low doses of chlorambucil. The fifth patient (W.S.) had recently received a course of oral cyclophosphamide and his serum IgM concentration and agglutinin and lysin titres fell during the period of investigation. One patient (E.B.) was deliberately exposed to a cold environment each day mid-way through his study, with resultant increase in intravascular haemolysis, to see the effect of this manoeuvre on his IgM catabolism.
TABLE 1. Clinical and laboratory data

<table>
<thead>
<tr>
<th>Subject</th>
<th>Age</th>
<th>Sex</th>
<th>Symptoms</th>
<th>Duration (years)</th>
<th>Hb (g/100 ml)</th>
<th>Reticulocytes (%)</th>
<th>Cold agglutinin titre at 4° v. adult 0 cells</th>
<th>Lysin titre at 20° v. papainized cells</th>
<th>Treatment at time of study (oral dose/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>E.B.</td>
<td>52</td>
<td>M</td>
<td>Severe haemolytic anaemia, Raynaud's phenomenon and haemoglobinuria</td>
<td>9</td>
<td>8.8</td>
<td>10.8</td>
<td>4000</td>
<td>640</td>
<td>Chlorambucil 2 mg</td>
</tr>
<tr>
<td>W.S.</td>
<td>69</td>
<td>M</td>
<td>Severe haemolytic anaemia, mild Raynaud's phenomenon</td>
<td>3½</td>
<td>10.3</td>
<td>8.0</td>
<td>8000</td>
<td>1280</td>
<td>Recent cyclophosphamide 200 mg</td>
</tr>
<tr>
<td>R.S.</td>
<td>56</td>
<td>M</td>
<td>Mild haemolytic anaemia, severe Raynaud's phenomenon</td>
<td>4</td>
<td>12.6</td>
<td>3.4</td>
<td>2000</td>
<td>160</td>
<td>Chlorambucil 4 mg</td>
</tr>
<tr>
<td>J.R.</td>
<td>71</td>
<td>F</td>
<td>Moderate haemolytic anaemia, mild Raynaud's phenomenon</td>
<td>5</td>
<td>11.8</td>
<td>2.1</td>
<td>32000</td>
<td>5000</td>
<td>Chlorambucil 2 mg</td>
</tr>
<tr>
<td>M.A.</td>
<td>84</td>
<td>F</td>
<td>Moderate haemolytic anaemia, occasional haemoglobinuria in the past</td>
<td>14</td>
<td>13.5</td>
<td>2.0</td>
<td>64000</td>
<td>5000</td>
<td>None</td>
</tr>
</tbody>
</table>
Controls

Three male and three female normal volunteers, aged 23–42 years, received the same iodinated cold agglutinins as the patients. In addition, a fourth male control received iodinated haemagglutinating 7S subunits. These controls were medical or technical staff working in a haematology laboratory and trained in the application of in vivo isotope techniques. They were aware of possible short-term or long-term hazards arising from the intravenous administration of radioiodinated cold agglutinins. Data from a study of cold agglutinin catabolism in a clinically normal 35-year-old female with coeliac disease under treatment with a gluten-free diet are included in Fig. 3.

Purification of cold agglutinins

The cold agglutinins were purified by the method described by Cooper (1968). The concentrated cold agglutinin eluates were passed through a column of Sephadex G-200 and each antibody appeared as a sharp peak at the exclusion volume of the column. 20–150 mg of cold agglutinin were recovered from each preparation. Evidence for the purity and electrophoretic homogeneity of the separated antibodies has been previously described (Cooper, 1968). Immunological studies showed that they were all type Kappa IgM immunoglobulins, and electrophoretic studies of the isolated light-chains indicated that the cold agglutinins were probably monoclonal.

Iodination of purified cold agglutinins

Iodination with $^{125}$I or $^{131}$I was performed by McFarlane's (1963) method, with modifications recommended by Helmkamp et al. (1967). Specific activities of 50–350 μCi/10 mg protein were obtained and the degree of iodination was controlled such that there was an average less than 0.5 atoms of iodine per cold agglutinin molecule. Iodinated cold agglutinins were at least 99.7% precipitable by TCA. Examination by cellulose-acetate electrophoresis showed that the radioactivity migrated as a sharply defined band with the same mobility ($\beta$–γ) as the cold agglutinin in the original serum.

IgM measurement

Total IgM concentrations in the sera of patients and controls were estimated by single radial immunodiffusion (Mancini et al., 1965). A concentrated pool of six purified cold agglutinins was used as an IgM standard.

Cold agglutinin 7S subunits

Haemagglutinating 7S subunits were prepared from the 'parent' 19S cold agglutinin of E.B. (Cooper, 1967) by reduction with 0.02 M cysteine (Miller & Metzger, 1965). These 7S subunits have intact sulphhydryl bonds between heavy and light chains.

TCA precipitability and antibody activity of injected cold agglutinins

During these studies serum samples from blood defibrinated at 37° were obtained from each subject. $^{125}$I and $^{131}$I radioactivity in the samples was at least 99.7% precipitable by TCA, and 70–93% of the radioactivity could be adsorbed onto human group-O red cells during a single incubation at 0°.
Protocol of experiments

Patients and controls received 180 mg of potassium iodide three times daily, commencing 2 days before and continuing throughout the study. 1–10 μg of cold agglutinin were injected intravenously and these amounts were labelled with not more than 30 μCi of 125I or 100 μCi of 131I respectively. The patients received their own 131I-labelled cold agglutinin (Table 3), and J.R., in addition, received 125I-labelled cold agglutinin EB. (Individual purified cold agglutinins are referred to by the letters of the patient's initials, e.g. patient E.B., cold agglutinin EB.) Controls 2 and 6 likewise received two cold agglutinins, one labelled with 125I, the other with 131I. Following intravenous injection of the labelled antibodies, blood samples were taken at frequent intervals on the first day, then once or twice daily until the study was completed. Continuous 24-hr urine collections were made on all five patients and on two controls. Total body counting was also carried out daily on two patients and two controls.

The radioactivity in plasma and urine samples was estimated in an autogamma scintillation counter against appropriate standards. Mixtures of 125I and 131I were separated by pulse-height analysis and corrections applied for 125I activity recorded at the 125I-photopeak. Total body radioactivity was measured in a shielded enclosure by a single uncollimated scintillation counter incorporating a 6-in. thallium activated sodium iodide crystal (Belcher, Anderson & Robinson, 1963).

Analysis of radioactivity data

Plasma radioactivity curves were resolved graphically into exponential functions and analysed by Matthew's (1957) method. Using this technique and accepting certain assumptions inherent in it (Freeman & Matthews, 1958), the following information was obtained:

(a) The fractional catabolic rate (FCR), i.e. the percentage of plasma radioactivity disappearing per day.

(b) The distribution of radioactivity between the intravascular and extravascular compartments.

(c) The synthesis (turnover) rates for IgM during steady state conditions, calculated by the following formula:

\[
\text{Synthesis rate (mg kg}^{-1}\text{ day}^{-1}) = \frac{\text{FCR} \times \text{Total intravascular IgM (mg)}}{\text{Weight (kg)}}
\]

In addition, the catabolic rate was calculated from the urinary excretion of radioactivity by the method of Pearson, Veall & Vetter (1958). The percentage of the original dose of radioactivity appearing in the urine per 24 hr (U) divided by the mean plasma radioactivity for that 24-hr period (P) remains approximately constant so that:

\[
\text{Catabolic rate (} %\text{/day}) = \frac{U}{P} \times 100
\]

The mean catabolic rate and the FCR should be similar if all the radioactivity is excreted in the urine and urine collection is complete.

In studies in which total body radioactivity was obtained, the distribution of radioactivity between the plasma and the extravascular space could be calculated by graphical calculation of the equilibrium time when total radioactivity in the extravascular compartment was at a maximum (Fig. 1). This extravascular radioactivity curve was obtained by subtracting the values on the plasma curve from the corresponding values on the total body curve.
Radiation (in rem) to whole body, blood and thyroid of patients and controls was calculated assuming; (a) a 20 day biological $T_+^*$, (b) blood to represent 10% of body weight, (c) a blocked thyroid to take up 1% of liberated iodine. These assumed values will tend to overestimate radiation exposure during the catabolism of radioiodinated IgM preparations.

On the basis of these assumptions and data for effective $\beta$- and $\gamma$-energies of $^{125}\text{I}$ and $^{131}\text{I}$ in blood, thyroid and whole body as an average (unpublished, Department of Medical Physics, Royal Postgraduate Medical School), then each 100 $\mu$Ci will give:

<table>
<thead>
<tr>
<th>Radiation (rem)</th>
<th>Whole body average</th>
<th>Blood</th>
<th>Thyroid</th>
</tr>
</thead>
<tbody>
<tr>
<td>$^{125}\text{I}$</td>
<td>0.10</td>
<td>0.4</td>
<td>3.0</td>
</tr>
<tr>
<td>$^{131}\text{I}$</td>
<td>0.20</td>
<td>1.9</td>
<td>1.4</td>
</tr>
</tbody>
</table>

Using these values, the radiation to patients and controls has been calculated as shown in Table 2.

**Table 2. Radiation to patients and controls**

<table>
<thead>
<tr>
<th>Subject</th>
<th>Wt (kg)</th>
<th>Iodine isotope administered</th>
<th>Radioactivity ($\mu$Ci)</th>
<th>Radiation dose (rem)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Whole body average</td>
<td>Blood</td>
</tr>
<tr>
<td>E.B.</td>
<td>65</td>
<td>131</td>
<td>30</td>
<td>0.06</td>
</tr>
<tr>
<td>W.S.</td>
<td>68</td>
<td>131</td>
<td>70</td>
<td>0.14</td>
</tr>
<tr>
<td>R.S.</td>
<td>96</td>
<td>131</td>
<td>70</td>
<td>0.10</td>
</tr>
<tr>
<td>J.R.</td>
<td>59</td>
<td>131</td>
<td>100</td>
<td>0.24</td>
</tr>
<tr>
<td></td>
<td></td>
<td>125</td>
<td>30</td>
<td>0.04</td>
</tr>
<tr>
<td>M.A.</td>
<td>65</td>
<td>131</td>
<td>100</td>
<td>0.22</td>
</tr>
<tr>
<td>1</td>
<td>87</td>
<td>131</td>
<td>40</td>
<td>0.06</td>
</tr>
<tr>
<td>2</td>
<td>50</td>
<td>131</td>
<td>40</td>
<td>0.11</td>
</tr>
<tr>
<td>3</td>
<td>66</td>
<td>131</td>
<td>100</td>
<td>0.21</td>
</tr>
<tr>
<td>4</td>
<td>57</td>
<td>131</td>
<td>20</td>
<td>0.05</td>
</tr>
<tr>
<td>5</td>
<td>54</td>
<td>131</td>
<td>30</td>
<td>0.08</td>
</tr>
<tr>
<td>6</td>
<td>61</td>
<td>131</td>
<td>25</td>
<td>0.06</td>
</tr>
<tr>
<td>*7</td>
<td>76</td>
<td>131</td>
<td>25</td>
<td>0.005</td>
</tr>
</tbody>
</table>

* A biological $T_+^*$ of 0.6 day was assumed for this study (see Results).

**RESULTS**

Fig. 1 illustrates the behaviour of $^{131}\text{I}$-labelled cold agglutinin EB injected intravenously into patient E.B. The plasma radioactivity fell progressively and the early curved part of the plasma slope (0–100 hr post-injection) could be resolved graphically into two exponential functions ($b_1$ and $b_2$). $b_2$ represents the flux of radioactivity into the extravascular compartment and $b_1$ its removal from the plasma due to catabolism of the cold agglutinin. After
equilibration with the extravascular compartment at 100 hr, plasma radioactivity declined as a single exponential ($b_1$) with a $T_1$ of 169 hr, indicating that catabolism of the labelled preparation from the plasma, or from some site in close proximity, continued at a constant fractional rate.

The total body radioactivity curve showed a slight initial inflection but then declined as a
### Table 3(a). Metabolism of cold agglutinins in patients with chronic cold haemagglutinin disease

<table>
<thead>
<tr>
<th>Subject</th>
<th>Cold agglutinin</th>
<th>Isotope</th>
<th>Plasma vol. (ml/kg)</th>
<th>Plasma survival (days)</th>
<th>Total body survival (days)</th>
<th>Intravascular component (%)</th>
<th>Fractional catabolic rate (%/day)</th>
<th>Catabolic rate (U/P ratio) (%/day)</th>
<th>Serum IgM concentration (mg/100 ml)</th>
<th>Total circulating IgM (mg)</th>
<th>IgM synthesis (turnover) rate (mg kg⁻¹ day⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>E.B.</td>
<td>EB</td>
<td>131I</td>
<td>48.8</td>
<td>7.0</td>
<td>7.0</td>
<td>78</td>
<td>13.2</td>
<td>12.8</td>
<td>220</td>
<td>7000</td>
<td>14.2</td>
</tr>
<tr>
<td>W.S.</td>
<td>WS</td>
<td>131I</td>
<td>51.5</td>
<td>6.2</td>
<td>—</td>
<td>70</td>
<td>18.1</td>
<td>20.7</td>
<td>135*</td>
<td>4580*</td>
<td>—†</td>
</tr>
<tr>
<td>R.S.</td>
<td>RS</td>
<td>131I</td>
<td>45.0</td>
<td>5.5</td>
<td>5.7</td>
<td>79</td>
<td>17.0</td>
<td>20.0</td>
<td>530</td>
<td>23050</td>
<td>41.5</td>
</tr>
<tr>
<td>J.R.</td>
<td>JR</td>
<td>131I</td>
<td>49.0</td>
<td>5.5</td>
<td>—</td>
<td>86</td>
<td>14.8</td>
<td>12.5</td>
<td>1020</td>
<td>29600</td>
<td>76.1</td>
</tr>
<tr>
<td>E.B.</td>
<td>EB</td>
<td>125I</td>
<td>50.4</td>
<td>5.5</td>
<td>—</td>
<td>86</td>
<td>14.8</td>
<td>13.4</td>
<td>630</td>
<td>21080</td>
<td>51.1</td>
</tr>
<tr>
<td>M.A.</td>
<td>MA</td>
<td>131I</td>
<td>42.5</td>
<td>7.4</td>
<td>—</td>
<td>83</td>
<td>11.5</td>
<td>9.8</td>
<td>1480</td>
<td>41200</td>
<td>72.5</td>
</tr>
<tr>
<td>Mean</td>
<td>—</td>
<td>—</td>
<td>47.6</td>
<td>6.3</td>
<td>—</td>
<td>80.3</td>
<td>14.9</td>
<td>15.1</td>
<td>677</td>
<td>21080</td>
<td>51.1</td>
</tr>
</tbody>
</table>

* Values at beginning of study.
† Not calculated; serum IgM concentration fell during study.
### Table 3(b). Metabolism of cold agglutinins in controls

<table>
<thead>
<tr>
<th>Control</th>
<th>Cold agglutinin</th>
<th>Isotope</th>
<th>Plasma vol. (ml/kg)</th>
<th>Plasma T&lt;sub&gt;1&lt;/sub&gt;-survival (days)</th>
<th>Total body T&lt;sub&gt;1&lt;/sub&gt;-survival (days)</th>
<th>Intravascular catabolic rate (％/day)</th>
<th>Catabolic rate from U/P ratio (％/day)</th>
<th>Serum IgM concentration (mg/100 ml)</th>
<th>Total circulating IgM (mg)</th>
<th>IgM synthesis rate (mg kg&lt;sup&gt;-1&lt;/sup&gt; day&lt;sup&gt;-1&lt;/sup&gt;)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>EB</td>
<td>&lt;sup&gt;131&lt;/sup&gt;I</td>
<td>40.0</td>
<td>7.6</td>
<td>8.1</td>
<td>85</td>
<td>12.3</td>
<td>10.3</td>
<td>148</td>
<td>5150</td>
</tr>
<tr>
<td>2</td>
<td>WS</td>
<td>&lt;sup&gt;131&lt;/sup&gt;I</td>
<td>48.1</td>
<td>5.6</td>
<td>—</td>
<td>76</td>
<td>17.6</td>
<td>—</td>
<td>112</td>
<td>2680</td>
</tr>
<tr>
<td>3</td>
<td>RS</td>
<td>&lt;sup&gt;131&lt;/sup&gt;I</td>
<td>40.5</td>
<td>6.4</td>
<td>6.6</td>
<td>76</td>
<td>14.9</td>
<td>14.8</td>
<td>113</td>
<td>2870</td>
</tr>
<tr>
<td>4</td>
<td>JR</td>
<td>&lt;sup&gt;131&lt;/sup&gt;I</td>
<td>42.6</td>
<td>5.0</td>
<td>—</td>
<td>74</td>
<td>19.4</td>
<td>—</td>
<td>78</td>
<td>1880</td>
</tr>
<tr>
<td>5</td>
<td>MA</td>
<td>&lt;sup&gt;131&lt;/sup&gt;I</td>
<td>41.3</td>
<td>7.9</td>
<td>—</td>
<td>84</td>
<td>10.8</td>
<td>—</td>
<td>128</td>
<td>3020</td>
</tr>
<tr>
<td>6</td>
<td>WS</td>
<td>&lt;sup&gt;131&lt;/sup&gt;I</td>
<td>44.0</td>
<td>4.7</td>
<td>—</td>
<td>85</td>
<td>17.3</td>
<td>—</td>
<td>60</td>
<td>1620</td>
</tr>
<tr>
<td>AC</td>
<td>&lt;sup&gt;125&lt;/sup&gt;I</td>
<td></td>
<td>45.4</td>
<td>5.0</td>
<td>—</td>
<td>79</td>
<td>16.7</td>
<td>—</td>
<td>—</td>
<td>2870</td>
</tr>
<tr>
<td>Mean</td>
<td>—</td>
<td></td>
<td>42.7</td>
<td>6.2</td>
<td>—</td>
<td>79·8</td>
<td>15·3</td>
<td>—</td>
<td>106</td>
<td>2870</td>
</tr>
</tbody>
</table>
single exponential almost parallel with the corresponding plasma slope. Radioactivity was therefore excreted at a constant fractional rate following its catabolism from the plasma. Exposure of patient E.B. to an ambient temperature of 8–12° for 2–3 hr daily during the mid-period of this study did not influence the catabolic rate.

Extravascular radioactivity rose to a maximum at 100 hr, then declined in parallel with the plasma and total body curves. At equilibrium, when there was no net exchange of radioactivity between the plasma and extravascular compartments, 81% was present in the plasma. This figure agrees well with the value of 78% distributed intravascularly, computed by Matthews' method. The fractional catabolic rate (FCR) was 13.2%/day and similar to the catabolic rate obtained from the mean daily U/P ratios, of 12.8%/day. These U/P ratios appear in the lower part of Fig. 1.

![Graph](https://via.placeholder.com/150)

**Fig. 2.** Plasma $^{131}$I (●) and $^{125}$I (○) radioactivity curves obtained from the study of patient J.R., who received $^{131}$I-labelled cold agglutinin JR and $^{125}$I cold agglutinin EB at the same time.

The results of the metabolic study on patient E.B. were representative of the results obtained in all the remaining studies. Tables 3(a) and 3(b) summarize the metabolic data of the patients and controls, respectively. The mean plasma volume was slightly higher in the patient groups (47.6 ml/kg) than in the control group (42.7 ml/kg) probably due to the presence of anaemic individuals among the patients. There is, however, a remarkably consistent pattern of distribution and catabolism of iodinated cold agglutinins when both groups are compared. Values for mean $T_1/2$ of 6.3 and 6.2 days, for mean FCR of 14.9 and 15.2%/day and for intravascular distribution of 80.3 and 79.8%, respectively, for patients and controls, were virtually identical. The catabolic rate for individual cold agglutinins, however, varied when studied in different
Cold agglutinin metabolism

For example, the FCR of cold agglutinin JR was 14.8%/day in patient J.R. and 19.4%/day in control 4, though when two different cold agglutinins were injected simultaneously into the same recipient, as in the case of J.R. and control 6, the catabolic rates for the two cold

![Graph](image)

**Fig. 3.** Plasma $^{125}$I (○) and $^{131}$I (●) radioactivity curves obtained from the study of control 6 (upper) and the patient with coeliac disease (lower). Each subject received two different cold agglutinins labelled with $^{125}$I and $^{131}$I respectively. Control 6 received cold agglutinins WS and AC and the patient with coeliac disease received cold agglutinins EB and MA.
agglutinins were virtually identical. Fig. 2 demonstrates that a single plasma radioactivity curve could be drawn for the two sets of data obtained when $^{125}$I EB and $^{131}$I JR were injected into J.R. Likewise, Fig. 3 shows two further examples, in which closely similar plasma radioactivity curves were obtained when two different $^{125}$I and $^{131}$I-labelled cold agglutinins were injected simultaneously into one subject (upper example, control 6, lower example, patient with coeliac disease).

![Plasma and total body $^{131}$I radioactivity curves obtained in the study of the control who received 7S haemagglutinating subunits derived from cold agglutinin EB.](image)

There was no correlation between the catabolic rate and the in vivo or in vitro haemolytic potency of the injected cold agglutinin, nor was there any correlation between the catabolic rate and the subject's plasma IgM concentration. There was no evidence from graphical resolution of the plasma radioactivity curves of minor compartments additional to the single extravascular pool, and inspection of the U/P ratios in individual studies showed no evidence of early elimination of a fraction of injected radioactivity which would indicate denaturation of the iodinated antibodies.

The patients' serum IgM concentrations ranged from 135 to 1480 mg/100 ml. Three patients,
R.S., J.R. and M.A., had 23, 30 and 41 g of circulating IgM, respectively, and adsorption studies have shown that most of this is cold antibody (Brown, D.L., Cooper, A.G. & Hepner, G.W., unpublished). IgM synthesis (turnover) rates calculated for the patients were 14.2–76.1 mg kg\(^{-1}\) day\(^{-1}\), the highest values being approximately ten times the corresponding control synthesis rates of 4.6–9.5 mg kg\(^{-1}\) day\(^{-1}\).

Fig. 4 shows the pattern of elimination of the haemagglutinating 7S subunits of cold agglutinin EB injected into a normal subject. Plasma and total body radioactivity fell rapidly. 48 hr after the injection only 5.7% of the radioactivity was still present in the plasma, though this remained precipitable by TCA and therefore protein-bound. Total body radioactivity had fallen at this time to 11%.

**DISCUSSION**

Two of the five patients with cold haemagglutinin disease were anaemic and showed overt haemolysis and all had very marked complement coating of their red cells resulting from the action of the cold antibodies *in vivo*. One patient (E.B.) was frequently exposed to a cold environment during his metabolic study and the degree of haemolysis increased. In spite of this variability in the clinical presentation and the known variability in the serological potency of the antibodies, no correlation was demonstrated between haemolysis *in vivo* and catabolic rates of the iodinated cold agglutinins. In addition, resolution of the plasma radioactivity curves by Matthews' method did not show any evidence of a third compartment, which might have occurred as a result of attachment and equilibration of the labelled cold agglutinins with the circulating red cells. Evans *et al.* (1968), however, have shown that interaction of cold agglutinins with pooled normal red cells *in vitro*, indicated by complement adsorption and lysis, may take place at temperatures as high as 37\(^\circ\). In addition to serological differences within this group of purified cold agglutinins, there were known differences in the light chain Inv allotypes and heavy-chain subgroups (Cooper, A.G., Franklin, E.C. & Steinberg, A.S., unpublished), in light chain amino acid composition and in electrophoretic mobility (Cohen & Cooper, 1968). Although fractional catabolic rates varied from 10.8 to 19.4%/day, this variation appeared to be primarily a characteristic of the recipient and not of individual cold agglutinins. Olesen & Hippe (1968) have recently suggested on the basis of cold agglutinin metabolic studies that normal IgM is metabolically heterogeneous in each subject. However, we observed that when two serologically, antigenically and chemically different cold agglutinins were injected simultaneously into the same individual, they were catabolized at virtually identical rates, i.e. they behaved as if they were metabolically homogeneous. Previous studies of the metabolism of normal IgM and of IgM derived from patients with Waldenström's macroglobulinaemia have suggested that homologous and autologous IgM may be catabolized at different rates (Barth *et al.*, 1964; Birke *et al.*, 1967) and in a recent report Jensen (1968) has concluded that a minor fraction is eliminated at an increased rate in the homologous recipient. In our studies, however, the mean catabolic rates in the patients (14.9%/day) and controls (15.3%/day) were virtually identical, indicating that taken as a group the homologous and autologous recipients treated the antibodies in the same way.

Our findings on the distribution and catabolism of cold agglutinins in our control group are similar to the data published by Barth *et al.* (1964) relating to normal IgM metabolism in normal subjects. Comparison with this and other reports on IgM metabolism (Birke *et al.*, 1967).
suggests that there is little or no difference between the metabolism of cold agglutinins and normal IgM.

The 'catabolic sites' therefore appear to react to some common structural feature of the cold agglutinin and IgM molecules, and this might be the constant portion of the \( \mu \)-chain or the assembled molecule. However, when biologically active \( 7S \) subunits, which retained their thermal agglutinating activity, were injected (Fig. 4), these were 'recognized' by the catabolic sites as abnormal and rapidly broken down. Olesen \& Hippe (1968) have previously reported very rapid catabolism of cold agglutinin subunits, though these were not held together by covalent bonds and lacked biological activity.

The present study has shown that in some cases as much as 20–40 g of cold agglutinin may be present in the plasma and 4–5 g synthesized and catabolized daily. In spite of this high turnover rate of abnormal immunoglobulin, little is known of the synthetic and catabolic sites or of the cells and control processes involved. The majority of patients have no demonstrable underlying neoplasm, no lymphadenopathy and no splenomegaly. Abnormal cells are as a rule not prominent in the bone marrow (Dacie, 1962), though Schubothe, Baumgartner \& Yoshimura (1961) have convincingly demonstrated increases in lymphoid cells in the marrow with the passage of time in a number of patients and the association of a minority of cases of cold haemagglutinin disease with lymphoma is well recognized. Van Furth \& Dieselloff den Dulk (1966) and Curtain \& Baumgarten (1965) localized IgM by fluorescent techniques to plasma cells in bone marrow and not to the lymphoid cells, though Marmont \& Damasio (1967) have recently demonstrated IgM fluorescence in abnormal lymphoid cells from a patient with cold agglutinin disease.

Three of the five patients in the present study were receiving, and one had recently received, alkylating agents in an attempt to reduce cold antibody concentrations and to control symptoms (Worlledge \textit{et al.}, 1968). The effect of alkylating agents on catabolic rates cannot be assessed in such a small group of patients, though the untreated patient (M.A.) had the longest \( T_1 \) survival (7.4 days) and the lowest fractional catabolic rate (11.5\%/day).

There is no evidence from the present study that the constant adsorption and elution of these cold antibodies or their participation in complement fixation cause sufficient molecular alteration to affect their metabolism.

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