The Direct Coombs Test in Lead Poisoning

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With the technical assistance of MARIE MINSTER

ONE PHASE of the anemia seen in clinical lead intoxication is attributed to an increase in the mechanical fragility of the erythrocytes and an increased rate of hemolysis. This change is produced by the chemical action of the lead salts on the external lipoprotein envelope of the erythrocytic membrane leading to microscopic denaturization with a loss of elasticity in the membrane. The transfusion of such cells into a normal recipient and the determination of their life span using the Ashby technic demonstrated that the damaged cells are removed from the circulation in a random manner and that the cellular defect was retained following transfusion into a normal recipient.

A second etiologic mechanism associated with the anemia of lead intoxication is the interference in the normal production of ferrous protoporphyrin. The blockade is detected by demonstrating an increase in the free protoporphyrin, coproporphyrin, and uroporphyrin in mature erythrocytes.

The present study is concerned with the alterations in the erythrocytic membrane seen in chronic lead intoxication in humans and in experimentally induced acute and chronic lead intoxication in dogs.

MATERIAL AND METHODS

Blood samples were collected from 29 workers with chronic asymptomatic lead intoxication at a local lead smelter. These individuals had been exposed to lead over a period of months or years and were selected for study because serial hematologic evaluations had revealed anemia or reticulocytosis. The control values for all human blood tests were obtained from a group of 20 normal hospital employees. The mongrel dogs used in the experiment were observed in quarantine for a period of one week and examined by a veterinarian to exclude disease. During the quarantine period each dog had two complete hemograms including the following: hemoglobin, hematocrit, erythrocyte and leukocyte count, basophilic stippling (fine and coarse), reticulocyte count, whole blood direct Coombs test, osmotic and mechanical fragility test, and the column Coombs Test (see below). The dogs were dewormed and inoculated for distemper and rabies. Purina Dog Chow supplemented by canned dog food was used except during the acute reaction phase of the lead poisoning. The solution of lead acetate used to induce poisoning in the dogs contained 0.5 Gm./6 ml. calculated as lead acetate. The salt was dissolved in normal saline and the pH was adjusted to 7.4 by adding sodium acetate. This final solution was filtered and the filtrate injected.

The hemoglobin was measured in an Evelyn colorimeter; the hematocrit was performed by the Wintrobe technic. The vital staining method as described by Whitby and Britton was used for counting reticulocytes. A modification of the method of McCord for measuring basophilic stippling was used as a vital staining technic and gave a higher value than that obtained by the dry film technic. All counting of reticulocytes and basophilic stippling was done using a phase microscope. The reticulocytes stained by the vital staining technic appear to be indistinguishable from the cells classified as showing coarse basophilic stippling.

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The fragility of both dog and human erythrocytes to mechanical trauma was measured by the method described by Shen and Castle. The osmotic fragility determinations were done utilizing the method of Parpart and Dacie.

The antiglobulin serum used in both the human and dog experiments was produced in the laboratory using whole serum rather than the gamma globulin fraction. The titer of both the human and dog antiglobulin sera reactions with an evaluation of the positivity of specific sera-cell reactions was checked by a second laboratory. The technic of the direct antiglobulin test applied to dog and human cells as described by Rosenfield was used throughout the study.

The life span of erythrocytes from normal and lead poisoned dogs was studied using radioactive chromium, Na₄Cr²⁴O₆. The cells were tagged in vitro and the compatibility for cross transfusion between dogs established by the indirect Coombs test prior to inducing lead intoxication. Four milliliter venous blood samples were collected periodically following transfusion. The life span of the tagged cells in the recipient animal was measured by relating the radioactivity due to Cr⁴¹ in each sample as counts per second per 4 ml. sample of whole blood as per cent of the radioactivity (c/s) found in the initial sample collected 24 hours after the injection of the tagged cell mass. The blood samples were hemolyzed to provide uniform geometry for counting in a well-type scintillation crystal. Corrections for the physical decay of the isotope were obviated by counting all the samples on the same day.

The disappearance rate of normal dog erythrocytes tagged in such a manner produces a curvilinear function similar to that found in the normal human if no corrections factor is applied for the possible elution of chromium.

**RESULTS**

Acute lead intoxication was produced in six dogs by injecting a lead acetate solution intravenously or into the peritoneal space. A local chemical inflammatory reaction was minimized by diluting the stock lead acetate solution in normal saline. This acute poisoning with lead produced a positive direct Coombs test within 24 hours in five of the six dogs. The sixth dog received a smaller dose of lead acetate intraperitoneally and developed a positive direct Coombs test after 13 days. The results of this acute experiment are seen in figure 1. The observations in most of the dogs were limited to 72 hours due to death of the animal. The acute intoxication with lead produced a clinical picture of generalized weakness, anorexia, diarrhea, peripheral motor paralysis, shock and death.

A state of chronic lead intoxication was produced in 11 dogs by the repeated intraperitoneal injection of small amounts of lead acetate solution. The dogs received a mean total dose of 1.4 Gm. of lead over a period of 19 days. The observations on the dogs were carried out for 50 days and the changes are described in table 1. Following the initial control period the animals all demonstrated a mild to moderate anemia with increased basophilic stippling and reticulocytosis. The dry film technic for demonstrating basophilic stippling was used in this particular experiment. There was an increase in the mechanical fragility. Although a definite alteration occurred in the mechanical fragility test in all dogs, there was no direct correlation with this test value and the degree of anemia. Similarly it was noted that there was no positive or negative correlation between the degree of alteration of mechanical fragility and the fine

* This evaluation and the initial supply of dog Coombs serum was obtained through the cooperation of Dr. E. E. Muirhead, Department of Pathology, Southwestern Medical School.

† Radioactive Pharmaceuticals Division, Abbott Laboratories, Oak Ridge, Tenn.
THE PRODUCTION OF A DIRECT POSITIVE COOMBS TEST IN DOGS USING LEAD ACETATE

TABLE 1.—Mean and Range of Hematologic Values on 11 Dogs with Lead-Induced Anemia

<table>
<thead>
<tr>
<th>Day</th>
<th>Hgb. (Gm./100 ml.)</th>
<th>Hct. (%)</th>
<th>Base. Stipp. (%)</th>
<th>Mech. Frag. (%)</th>
<th>WBC</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>14.5 (11.3-17.9)</td>
<td>43.1</td>
<td>0.9 (0.4-1.6)</td>
<td>3.7 (1.8-5.4)</td>
<td>13.8</td>
</tr>
<tr>
<td>10</td>
<td>13.8 (9.6-16.9)</td>
<td>40.9</td>
<td>1.4 (0.6-3.9)</td>
<td>8.0 (3.2-18.2)</td>
<td>26.2</td>
</tr>
<tr>
<td>20</td>
<td>12.7 (9.2-15.4)</td>
<td>38.9</td>
<td>2.0 (0.5-7.6)</td>
<td>7.0 (2.7-16.7)</td>
<td>22.7</td>
</tr>
<tr>
<td>30</td>
<td>11.6 (7.0-14.5)</td>
<td>34.9</td>
<td>1.2 (0.5-2.2)</td>
<td>6.1 (1.2-15.0)</td>
<td>24.3</td>
</tr>
<tr>
<td>40</td>
<td>10.3 (6.6-14.0)</td>
<td>32.6</td>
<td>2.6 (0.8-3.8)</td>
<td>8.8 (2.4-18.8)</td>
<td>—</td>
</tr>
<tr>
<td>50</td>
<td>10.4 (6.2-12.8)</td>
<td>31.8</td>
<td>10.5</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

basophilic stippling or the positivity of the direct Coombs test. A leukocytosis occurred as the lead intoxication progressed. No infection was found at autopsy that could have been responsible for a leukocytosis. There was a slight perihepatitis and perisplenitis as well as mild generalized thickening of the peritoneum at autopsy. This was interpreted as a chemical peritonitis due to the lead subacetate solution. There was no exudate on the surface of the organs nor was there any ascites at autopsy.

The development of the positive direct Coombs test in all 11 dogs given a more chronic lead poisoning is shown in figure 2. A positive reaction occurred in many instances within the first five days and 4 plus reaction was induced as early
as 13 days. A positive direct Coombs reaction has been found up to 62 days following lead intoxication. The animals developed lassitude, anorexia, weight loss, diarrhea, and occasionally a mild motor paralysis extending over a period of three weeks. Following this three week period the dogs appeared normal except for a persistent mild anorexia and associated weight loss.

During the chronic lead intoxication experiment it was noted that blood drawn from the poisoned dogs did not settle out promptly into two clearly defined portions of erythrocytes and supernatant plasma but that a column of erythrocytes remained suspended in the plasma. In the blood samples that showed the greatest positivity of the direct Coombs test it was observed that the supernatant cloud of erythrocytes was most marked and that it persisted in vitro over a 24 hour period. The changes in the erythrocytes produced by lead poisoning in respect to their sedimenting property was then investigated by placing the blood in plastic tubing held in the vertical position and sampling the column at various levels by puncturing the tubing with a small needle and aspirating a segment of the column. Complete hematologic studies including the direct Coombs test was done on each of three or four samples taken at equally spaced intervals over the column as well as on a sample of the whole blood. The direct Coombs test was uniformly more positive on the cells in the supernatant cell fraction than in any other level tested. In some samples the supernatant cell fraction was 3 plus and the whole blood sample had a negative reaction. Analysis done on the same samples from the column showed that coarse basophilic stippling was also predominantly in the superior portion of the column. Fine basophilic stippling was found at all levels in the column with a slight preponderance in the top one-third. These observations were repeated in 12 normal dogs and in only one dog was there a one+ positive direct Coombs test obtained from
the superior portion of a packed column of erythrocytes or from a partially sedimented column of erythrocytes. This dog was normal by physical examination and by examination of the peripheral blood. There was however a reticulocytosis of 23 per cent on smear of the top layer of the sedimented column. The incidence of the coarse basophilic stipple cell count was usually the same as the reticulocyte count if both were done by the vital staining methods. These findings are summarized in table 2.

A similar study was carried out on blood samples from twenty normal humans and in all samples the direct Coombs test was negative at all levels of the column. These data are shown in table 3.

The whole blood samples from 10 workers chronically exposed to lead were found to have a negative direct Coombs test. An additional 19 workers were studied using both the whole blood reaction and the column analysis. These men were asymptomatic and blood samples were obtained at the time of their usual monthly examination. Seventy-nine per cent of the superior portions of the columns revealed a positive direct Coombs test and in all samples this test was negative on the whole blood and the inferior portion of the column. The coarse basophilic stippling count and reticulocyte count in the different fractions of the columns closely paralleled the positivity of the Coombs test. This gradation of values on the columns is shown in table 4. The number of erythrocytes remaining suspended in the supernatant plasma did not approach that seen in the poisoned dogs and in some blood samples there was no visible fraction

### Table 2. Hematologic Findings in 12 Normal Dogs

<table>
<thead>
<tr>
<th></th>
<th>Coombs Test</th>
<th>Coarse Stippling</th>
<th>Light Stippling</th>
<th>Reticulocyte Count</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ave. Range</td>
<td>Ave. Range</td>
<td>Ave. Range</td>
<td>Ave. Range</td>
</tr>
<tr>
<td>Part I</td>
<td>0.2+ Neg. to 1+</td>
<td>9.2 1-20</td>
<td>5.0 1-21</td>
<td>7.9 1.2-23.1</td>
</tr>
<tr>
<td>Part II</td>
<td>Neg. Neg.</td>
<td>1.6 0-7</td>
<td>3.1 1-8</td>
<td>2.8 0.8-5.9</td>
</tr>
<tr>
<td>Whole Blood</td>
<td>Neg. Neg.</td>
<td>1.3 0-3</td>
<td>2.4 0-7</td>
<td>1.6 0.4-3.8</td>
</tr>
</tbody>
</table>

### Table 3. Hematologic Findings in 20 Normal Humans

<table>
<thead>
<tr>
<th></th>
<th>Coombs Test</th>
<th>Coarse Stippling</th>
<th>Light Stippling</th>
<th>Reticulocyte Count</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ave. Range</td>
<td>Ave. Range</td>
<td>Ave. Range</td>
<td>Ave. Range</td>
</tr>
<tr>
<td>Part I</td>
<td>Neg.</td>
<td>1.30 0-4</td>
<td>6.1 2-12</td>
<td></td>
</tr>
<tr>
<td>Part II</td>
<td>Neg. 0.67</td>
<td>0.2 0-2</td>
<td>5.5 1-10</td>
<td></td>
</tr>
<tr>
<td>Whole Blood</td>
<td>Neg. 1.30</td>
<td>0-3</td>
<td>4.5 1-7</td>
<td>1.6 0.9-3.9</td>
</tr>
</tbody>
</table>

### Table 4. Hematologic Findings in 19 Men with Chronic Lead Intoxication

<table>
<thead>
<tr>
<th></th>
<th>Coombs Test</th>
<th>Coarse Stippling</th>
<th>Light Stippling</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ave. Range</td>
<td>Ave. Range</td>
<td>Ave. Range</td>
</tr>
<tr>
<td>Part I</td>
<td>1.2+ Neg. to 2+</td>
<td>9.8 3-31</td>
<td>44.8 23-84</td>
</tr>
<tr>
<td>Part II</td>
<td>0.4+ Neg. to 1+</td>
<td>4.3 0-11</td>
<td>31.5 9-66</td>
</tr>
<tr>
<td>Whole Blood</td>
<td>Neg. Neg. to ±</td>
<td>3.5 0-13</td>
<td>16.9 7-66</td>
</tr>
</tbody>
</table>
of erythrocytes suspended in the supernatant plasma. In these latter samples the topmost portion of erythrocytes was chosen when the cells had begun to sediment into a partially formed column.

One hundred ml. of heparinized blood were drawn from one of the dogs whose blood showed a marked column effect in which the supernatant fraction had a high coarse stippling count and a 4+ direct Coombs test. The sample was suspended in many plastic columns and the superior and inferior fractions of the columns removed and pooled. The two fractions of erythrocytes were then tagged with radioactive chromium and transfused into compatible recipient normal dogs. The cells from the superior portions of the columns had a markedly shortened life span of approximately ten days. The cells pooled from the inferior portions of the columns had a mean life span of 40 days. The mean life span of normal dog erythrocytes determined by the radioactive chromium technic and calculated by the extrapolation of the initial phase of the curvilinear decay function approximates 70 days with an average total extinction point at 115 days. These decay curves are illustrated in figure 3.

**Discussion**

The basic studies recorded in 1925 by Aub clearly demonstrated that lead poisoning produced chemical and physical changes in the matrix of the erythro-
The altered membrane could produce a disruption of the normal lipoprotein membrane in such a way as to expose or project globulin groups to the surface. Such an alteration in structure would then be detected by the anti-globulin test of Coombs. The possibility that globulin is precipitated onto the erythrocyte from the plasma proteins at the time of the damage to the membrane should be considered.

The work of McFadzean has shown that the toxic effect of lead on erythrocytes begins in the marrow. Basophilic stippling was shown to occur as hemoglobin synthesis began but not prior to such chemical differentiation. The positive antiglobulin test in both humans and dogs suffering from lead intoxication has correlated closely with the reticulocyte or coarse basophilic stippling value. The two possible conclusions that may be drawn from the correlation are as follows: (1) reticulocytes retain free globulin groups on the membrane causing a positive direct Coombs test, or (2) immature erythrocytes are preferentially damaged by lead and the chemical reaction on the cell membrane causes the alteration. If the former hypothesis explains the observed changes, it will be a factor to be evaluated in any case of severe hemolysis where the erythrocytic hyperplasia of the marrow causes a marked release of immature cells into the peripheral blood.

**Summary and Conclusions**

1. The anemia produced by lead intoxication in humans as well as experimentally induced lead poisoning in dogs results in a positive direct Coombs test.
2. The direct antiglobulin test will become positive within twenty-four hours in cases of severe lead poisoning produced experimentally in dogs.
3. The blood from cases of severe lead poisoning in dogs will form a layer phenomenon when allowed to stand with the formation of a supernatant fraction of cells above the packed erythrocytes. These cells remain suspended in the plasma for many hours. This superior fraction on a column of blood has a high per cent of reticulocytes, and cells with coarse basophilic stippling. The direct Coombs test is positive in the superior fraction of such a column. The positivity decreases as the sampling approaches the bottom. In many instances the whole blood direct Coombs test may be negative and the cells from the superior portion of the column be strongly positive. The latter phenomenon was also found in asymptomatic workers exposed to lead in a smelting plant.
4. The possible significance of the correlation of the direct anti-globulin reaction and cell immaturity or chemical trauma to the membrane is briefly discussed.

**Summario in Interlingua**

1. Le anemia resultante de intoxication a plumblo in humanos etiam le anemia resultante de saturnismo experimental in canes effectua un positive reaction del directe test de Coombs.
2. Le directe test a antiglobulina deveni positive intra vinti-quatro horas in casos de sever saturnismo de production experimental in canes.
3. Le sanguine ab casos de sever saturnismo in canes exhibi un phenomeno de stratification que se declara post un certe periodo de tranquillitate, con le
formation de un supernatante fraction de cellulas supra le paccate eryt-rocytos. Iste cellulas remaine suspendite in le plasma durante multe horas. Iste fraction superior in le columna de sanguine ha un alte procentage de reticulocytes e cellulas con grossier marmorisation basophilie. Le directe test de Coombs es positive in le fraction superior de un tal columna Le positivitate decresce in specimenz prendite plus proximie al fundo. In multe casos le directe test de Coombs con sanguine integre pote esser negative durante que le cellulas in le portion superior del columna monstra un reaction fortemente positive. Iste ultime phenomeno esseva etiam observate in le caso de obreros asymptomatic qui esseva exponite a plumbo in un funderia.

4. Es presentate un breve diseussiomi del possihile signification del correlation inter le directe reaction a antiglobulina e le immaturitate cellular o le trauma chimic in le membrana.

REFERENCES

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