Sulfhemoglobinemia and Acute Hemolytic Anemia with Heinz Bodies Following Contact with a Fungicide—Zinc Ethylene Bisdithiocarbamate—in a Subject with Glucose-6-Phosphate Dehydrogenase Deficiency and Hypocatalasemia

By Jack Pinkhas, Meir Djaldetti, Henry Joshua, Chaim Resnick and André de Vries

The discovery in 1934 by Tisdale and Williams of the fungitoxicity of derivatives of dithiocarbamic acid introduced one of the most versatile groups of fungicides. The important members of these fungicides fall into three classes; the metallic dithiocarbamates, the thiuram sulfides and the bisdithiocarbamates. The bisdithiocarbamates of practical importance are the disodium, the manganous and zinc salts of ethylene bisdithiocarbamic acid known as nabam, maneb and zineb.

In the present report we wish to describe a patient in whom sulfhemoglobinemia and acute hemolytic anemia with Heinz body formation appeared following contact with the latter fungicide—zinc ethylene bisdithiocarbamate (fig. 1)—which, although widely used in Israel in crop protection, had until now not been reported to produce undesirable effects in the human. This patient was remarkable in having, in addition to glucose-6-phosphate dehydrogenase deficiency, severe hypocatalasemia.

The combination of sulfhemoglobinemia and hemolytic anemia, apparently, is rare and has been reported in the literature in a very few cases only. A predisposing factor to the hemolysis in our patient may have been the low glucose-6-phosphate dehydrogenase activity of his red blood cells, known to be involved in hemolysis following exposure to various other drugs, such as primaquine and sulfonamides, and to fava bean. The fungicide, presumed to have caused the sulfhemoglobinemia and hemolytic anemia in this patient, was shown in vitro to produce Heinz bodies and to reduce the glutathione content of his red blood cells, as well as of those of other individuals with unstable red blood cell glutathione. In addition, the possibility that the severe catalase deficiency of the patient's red blood cells played a role in the causation of both the hemolysis and the sulfhemoglobinemia is considered.

Methods

Standard hematologic and serologic methods were used as described by Dacie. The glutathione content and stability of the red blood cells were determined by the methods described by Gruenert and Phillips, as modified by Beutler, the glucose-6-phosphate dehydrogenase activity was estimated according to the method of Marks and the catalase

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\[
\text{CH}_2 - \text{NH} - \text{C} - \text{S} \quad \text{Zn} \\
\text{CH}_2 - \text{NH} - \text{C} - \text{S} \quad \text{S}
\]

Fig. 1.—Structure of zinc ethylene bisdithiocarbamate.

activity according to Tarlov and Kellermeyer. Examination for Heinz bodies was done on a wet-slide preparation, using Nile blue sulfate 0.5 per cent in absolute alcohol.

Sulfhemoglobin and methemoglobin in the red blood cells and the plasma were determined by the methods of Cartwright and Dacie, respectively. Urine porphobilinogen was searched for by the method of Watson and Schwartz and urine porphyrins were determined according to the method of Sveinsson et al. P-nitrophenol, indicative of parathion poisoning, was searched for in the urine by the chromatographic method of Eichhorn and Menulhin-Lifschitz, thallium according to the procedure described by Rappaport and Eichhorn and arsenic by the Gutzeit test as described by Simmons.

CASE REPORT

G. J., a 32-year old Persian-born Jewish farmer who immigrated to Israel in 1958, father of five children, was admitted to the medical department in April 1961. His previous history was negative, except for malaria in 1956. He had no history of inflammation or ulceration in the mouth. No diseases were known in his family.

On the day of admission he had started to work in the field early in the morning and felt well until about noontime, when he complained of headache and fever. He left his work and received 2 tablets of A.P.C. (aspirin 250 mg., phenacetin 250 mg., caffeine 50 mg.), but his condition worsened, his temperature rose to 40 C. and in the afternoon he noticed dark urine. Inquiry revealed that the field in which he had been working had been sprayed during the early hours of the morning with a fungicide, and that he had taken his breakfast at 9 o’clock in the morning while resting in the field without cleaning his hands. He had not eaten fava beans.

Physical examination showed the patient in a severe condition with a peculiar yellowish and slate-blue color of the skin and sclerae. The earlobes and the fingertips showed a greyish-blue tinge. His temperature was 39 C., the blood pressure 135/80 mm. Hg, the pulse rate 100 per minute, the respiratory rate 28 per minute. There were no abnormal findings in the mouth. The spleen and liver were not palpable. Neurologic examination was normal. His urine was dark brown, albumin was negative, the sediment showed no formed elements, bilirubin was absent, urobilinogen (by the qualitative Ehrlich reaction) was not increased, porphobilinogen and uroporphyrin were absent, coproporphyrin was 2.4 mg. per cent. P-nitrophenol (parathion poisoning), thallium and arsenic were not found. Blood hemoglobin was 12.3 Gm. per cent; the red blood cells could not be counted since many of them were destroyed. The peripheral blood smear showed marked anisocytosis, anisochromia and deformed red blood cells, in some the hemoglobin apparently taking up only a part of the cell. Heinz bodies were seen in numerous red cells in the wet-slide preparation (fig. 2). Reticulocytes could not be counted in the vital-stain preparation. The white blood cell count was 17,000/cu.mm. with 59 per cent neutrophils, 6 per cent band forms, 2 per cent basophils, 20 per cent lymphocytes and 13 per cent monocytes. The platelet count was 122,000/cu.mm., the bleeding time 1 minute, the clotting time 4 minutes. A bone marrow needle biopsy showed normoblastic hyperplasia. The direct and indirect Coombs tests and the Ham and Crosby tests were negative. The red blood cell glutathione content was 11 mg./100 ml. packed red blood cells and, after 2 hours incubation at 37 C. with acetylphenylhydrazine, was 9 mg./100 ml. packed red
blood cells. The serum bilirubin was 4.2 mg. per cent, of that indirect-reacting 3.4 mg. per cent, the serum iron 200 μg. per cent. Other routine blood chemistry values were normal, including the serum paper electrophoretic protein pattern.

On the day following admission he became semicomatose, the cyanosis increased and was not improved by oxygen administration. The urine was dark brown and contained sulfhemoglobin. A venous blood sample had a dark brown color which did not disappear after shaking with oxygen and was found to contain sulfhemoglobin but no methemoglobin. Prior to this laboratory report, methylene blue and vitamin C had been given intravenously without improvement. His hemoglobin was 10.8 Gm. per cent.

On the fourth day his condition became critical and the hemoglobin had dropped to 4.7 Gm. per cent with a red blood cell count of 1,700,000 per cu.mm. Six units of blood, two of these as packed cells, and 300 mg. cortisone, intramuscularly, were given. Improvement was dramatic, his consciousness became clear, the cyanosis decreased markedly and his pulse and respiratory rate returned to normal. On the following day the blood had gained a red color, the hemoglobin was 9.7 Gm. per cent, the red blood cell count 3,100,000 per cu.mm., the reticulocyte count 7.6 per cent. Heinz bodies were still found in the red blood cells, but much less than on admission. The platelet count was 85,000 per cu. mm., but there were no hemorrhages. The urine gradually regained a normal color and the serum bilirubin was normal. The patient was discharged 2 weeks after admission and requested to return for further investigation after 3 months.

In July 1961, he was in a good condition, his hemoglobin was 12.6 Gm. per cent, the red blood cell count 4,200,000 per cu.mm., reticulocytes 0.56 per cent, no Heinz bodies were demonstrated, the white cell count was 6,900 per cu.mm., the platelet count 189,000 per cu.mm. The red cell glucose-6-phosphate dehydrogenase was 1.2 units per Gm. hemoglobin (normal = 10–20 units), the reduced glutathione was 42 mg. per 100 ml packed red blood cells and, after incubation, with acetylphenylhydrazine, 18 mg. per 100 ml packed cells.

Investigation of an vitro action of the fungicide and APC on the red blood cells of the patient and of other glutathione-instable "healthy" subjects was undertaken. Incubation of patient’s washed red cells (obtained in July 1961 when healthy) with a one per cent suspension of APC in saline for 12 hours at 37 C. did not produce Heinz bodies. Incubation of patient's whole blood or patient's washed red blood cells with fungicide suspension at a final concentration of 5 mg./ml. for 12 hours at 37 C. caused the appearance of Heinz bodies (table 1). Similar results were obtained with blood or washed red cells of a "healthy" nonanemic Yemenite Jew who had unstable red cell glutathione. On the other hand, the fungicide did not induce Heinz body formation in
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Table 1.—Appearance of Heinz Bodies in Patient’s Red Blood Cells Following Incubation with the Fungicide for 12 Hours at 37°C.

<table>
<thead>
<tr>
<th>Patient 3 Months after Recovery</th>
<th>Healthy Subject with Stable Red Cell Glutathione*</th>
<th>Healthy Subject with Instable Red Cell Glutathione†</th>
<th>Fungicide‡ (10 mg/ml.)</th>
<th>Heinz Bodies</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>+</td>
</tr>
<tr>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>+</td>
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<tr>
<td>0.5</td>
<td>0.5</td>
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<tr>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>+</td>
</tr>
</tbody>
</table>

*Red cell glutathione = 70 mg. per 100 ml. packed red cells; after incubation with acetylphenylhydrazine = 67 mg. per 100 ml.
†Red cell glutathione = 38 mg. per 100 ml. packed red cells; after incubation with acetylphenylhydrazine = 6 mg. per 100 ml.
‡Zinc ethylene bisdithiocarbamate.
§All controls without fungicide (made up with saline) negative.

Table 2.—Erythrocyte Glutathione Stability Test with the Fungicide and Acetylphenylhydrazine

<table>
<thead>
<tr>
<th>Source of Red Blood Cells</th>
<th>Glutathione, mg. per 100 ml. Packed Blood Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before incubation</td>
</tr>
<tr>
<td>Patient 3 months after recovery</td>
<td>42</td>
</tr>
<tr>
<td>Healthy Yemenite Jews</td>
<td>38</td>
</tr>
<tr>
<td></td>
<td>40</td>
</tr>
<tr>
<td></td>
<td>37</td>
</tr>
<tr>
<td>Healthy Ashkenazi Jews</td>
<td>38</td>
</tr>
<tr>
<td></td>
<td>70</td>
</tr>
<tr>
<td></td>
<td>60</td>
</tr>
<tr>
<td></td>
<td>80</td>
</tr>
</tbody>
</table>

System: 1 ml. blood in ACD (1:10) + 5 mg. acetylphenylhydrazine or 5 mg. zinc ethylene bisdithiocarbamate, incubated for 2 hours at 37°C.

The blood from a healthy Ashkenazi Jew whose red cell glutathione was stable. No activity, neither inhibiting nor promoting the Heinz body formation, was detected in the sera of these individuals.

It will be seen from table 2 that the fungicide was able to reduce markedly the glutathione content of the patient’s red blood cells, and of “healthy” Yemenite Jews with known glutathione instability, similar to the action of acetylphenylhydrazine. On the other hand, the slight decrease brought about by the fungicide in the glutathione of the red blood cells from healthy Ashkenazi subjects, known to be glutathione-stable (table 2) was not greater than that produced by acetylphenylhydrazine. Sulfhemoglobin was not detected following incubation of the red blood cells from the patient or from other glutathione-instable individuals with the fungicide.

In September 1962, in the search for an additional factor possibly underlying the sensitivity of the patient to the fungicide, his red blood cell catalase activity was examined and found to be extremely low—0.3 x 10⁻¹⁹ moles per red blood cell, which is about 6 to 7 per cent of normal (4.05 – 5.25 x 10⁻¹⁹, mean 4.65). At this time the patient...
was healthy, not anemic, and had no contact with the fungicide and indeed had discontinued to work with all pesticides. Repeated examinations, 2 and 4 weeks later, gave similar results—0.3 \(^{-19}\) and 0.41 \(^{-19}\) moles per red blood cell, respectively.

An extensive study of the patient’s family is being undertaken but preliminary investigation indicated the hypocatalasemia to be a hereditary condition with an autosomal mode of transmission and intermediate catalase activity values in heterozygotes (two sons and two daughters of the patient). The term hypocatalasemia has been used by the Japanese investigators for the heterozygotes with intermediate catalase values, whereas the homozygotes were designated as acatalasemic.\(^{15}\) In contradistinction we have applied the term hypocatalasemic to our patient, presumed to be homozygous for the enzymatic defect, to indicate the presence of a small amount of catalase activity in his red blood cells. It should be noted, however, that a slight catalase activity was also recorded\(^{16,17}\) in the red blood cells of some of the Japanese and Swiss acatalasemic subjects.

**DISCUSSION**

According to Finch\(^{18}\) 30 to 50 per cent of the hemoglobin may be replaced by methemoglobin without producing clinical symptoms, and Tepperman et al.,\(^{19}\) reported in working subjects with paraaminopropiophenone-induced methemoglobinemia complaints of mild fatigue only at a methemoglobin level of 20 per cent. In sulfhemoglobinemia, cyanosis occurs already when about 0.5 Gm. per cent of the hemoglobin has been replaced by the inert pigment,\(^{20}\) thus serving an early warning sign of intoxication. It is understandable, therefore, why sulfhemoglobinemia per se rarely produces severe clinical manifestations, and death from sulfhemoglobinemia seems to be unusual.\(^{21}\) Although the sulfhemoglobin level was not determined in our patient, it is probable that his severe condition was chiefly due to the acute hemolytic anemia, the anoxia being aggravated by the sulfhemoglobinemia.

The mechanism by which the fungicide, zinc ethylenebisdithiocarbamate, produced the sulfhemoglobinemia is not known. It is probable that the high sulfur content of this chemical (fig. 1) played a role, since Harrop and Waterfield were able to produce sulfhemoglobinemia in dogs (splenectomized) by feeding them with sulfur.\(^{22}\) It is pertinent that zinc ethylene bisdithiocarbamate has been shown to decompose to ethylene thiourea, carbon disulfide and hydrogen sulfide both under slightly acid and under alkaline conditions (fig. 3).\(^{23}\) Possibly the hydrogen sulfide was responsible for the sulfhemoglobinemia in our patient. It can not be excluded that a role may also be ascribed to the nitrogen present in the molecule in hydrogenated form, in view of the experiments reported by Silver\(^{24}\) who produced sulfhemoglobinemia in dogs and rabbits by administration of precipitated sulfur together with acetophenetidin, TNT or para-amino-propriophenone. In this context a potentiating effect of the acetophenetidin taken by the patient after exposure to the fungicide may also be considered.

It is of interest that in four out of nine patients who had sulfhemoglobinemia, McCutcheon\(^{25}\) found increased reduced glutathione levels in the blood, in two of them at the time when the pigment was not demonstrable any more. He postulated that a high reduced glutathione level may lead to increased production of oxidized glutathione, which in turn may liberate hydrogen sulfide, such a reaction being enhanced by various drugs. A sensitivity due
to such a mechanism was certainly not involved in our patient, since his blood was characterized by a low reduced glutathione concentration.

Finally, the possibility may be considered that the hypocatalasemia was instrumental in promoting the sulfhemoglobin formation in this patient, since, according to the work of Deul, the production of sulfhemoglobin from crystalline ox hemoglobin by the disulfhydryl compound, 2-3-dimercapto-propanol (BAL), is inhibited by catalase.

In the explanation of the hemolytic anemia occurring in our patient following contact with the fungicide, two factors must be taken into consideration: the enzymatic defects of his red blood cells and the quantity of the toxic material taken up.

Glucose-6-phosphate dehydrogenase deficiency with low and unstable reduced glutathione of the red blood cells is known to be involved in the sensitivity to hemolysis induced by sulfonamides, primaquine, nitrofurantoin, naphthalene derivatives and fava bean. Our patient belongs to a group of subjects, Persian Jews, in whom red blood cell glutathione instability is prevalent. That glutathione instability is implicated in the hemolysis in our patient seems probable since incubation of his red blood cells with the fungicide decreased their glutathione content markedly, similar to the effect of acetylphenylhydrazine. Furthermore, the Heinz body formation in our patient’s red blood cells on contact with the fungicide both in vivo and in vitro fits well with the Heinz body formation known to occur in sensitive glutathione unstable red blood cells when acted upon by various chemicals. Finally, the fungicide was able to lower the glutathione concentration and induce Heinz body formation in the red blood cells of other subjects with known
glutathione instability. It is pertinent that Chefurka found ethylene thiuram monosulfide, which is a decomposition product of metal bisdithiocarbamates (fig. 3), to be a potent inhibitor of glucose-6-phosphate dehydrogenase in the housefly. Moreover, it has been established by Sijpesteyn and van der Kerk that isothiocyanate groups, which are intermediates in the formation of ethylene thiuram monosulfide from the fungicide, are strong inhibitors of sulfhydryl-containing enzymes.

One difficulty in relating the hemolytic anemia in our patient following contact with the fungicide to the enzymatic red blood cell defect is that, to the best of our knowledge, until now no similar cases have been reported in this country nor in other countries, although glucose-6-phosphate dehydrogenase deficiency is prevalent and the fungicide is widely applied, the yearly amount used in Israel being about 200,000 kilograms. It is possible that there were many undetected "subclinical" cases of hemolysis in such "sensitive" individuals. On the other hand, the quantity of fungicide which was absorbed into the patient's body may have been exceptionally large. He took his meal while resting in the field, without having cleaned his hands, with the food having been in direct contact with the sprayed crop. Finally, it may be asked whether the hypocatalasemia contributed to the sensitivity of patient's red blood cells to the hemolytic action of the fungicide. Tarlov and Kellermeyer found slightly decreased catalase activity (60-80 per cent of normal) in hemolysates from primaquine-sensitive individuals prior to drug administration and a sharp drop during hemolysis, representing a fall of not more than 35 per cent of the initial value. These authors suggested that low catalase activity may render the red blood cells of intermediate glucose-6-phosphate dehydrogenase deficient females more susceptible to hemolysis. Whereas the role of this slight decrease in catalase activity in drug-induced hemolysis has not yet been clarified, the severe hypocatalasemia (6-10 per cent) in our patient may possibly have greater significance. Takahara found that red blood cells from subjects with acatalasemia are unusually susceptible to methemoglobin formation on exposure to hydrogen peroxide, the latter according to various investigations being an essential stage preceding the destruction of erythrocytes precipitated by primaquine and similarly acting oxidant drugs. Drug-induced hemolytic anemia has not been reported in the Japanese and Swiss acatalasemic subjects, but neither is information available on whether among them there are glucose-6-phosphate dehydrogenase deficient individuals. Possibly, the joint occurrence of both defects, i.e., glucose-6-phosphate dehydrogenase deficiency and acatalasemia or severe hypocatalasemia such as in our patient, may be required for full expression of the sensitivity to specific agents such as the zinc ethylene bisdithiocarbamate.

There is no evidence in the literature that sulfhemoglobin itself is a cause of accelerated red cell destruction. On the contrary, the experiments of Silver et al. indicated that the rate of hemolysis and the degree of sulfhemoglobin formation induced by p-aminopropiophenone, acetophenetidin and TNT are unrelated. The hemolysis and the sulfhemoglobinemia in our patient therefore may be considered coincident but mutually independent effects of the
zinc ethylene bisdithiocarbamate, producing the former by way of ethylene-
thiuram monosulfide and the latter by way of formation of hydrogen sulfide.

Sulfhemoglobin, when appearing in the red blood cells following contact
with drugs or chemicals, persists during the whole red blood cell life span\textsuperscript{41,42}
and, in contradistinction to methemoglobin, is not reversed to hemoglobin by
reducing agents, such as ascorbic acid\textsuperscript{43} or by the redox dye methylene blue.\textsuperscript{44}
For severe sulfhemoglobinemia with levels so high as to endanger the pa-
tient's life by anoxia, exchange transfusion has been advocated.\textsuperscript{45} When hemo-
lytic anemia is present, as in the described case, blood transfusion and steroids
should be given. Since, however, sulfhemoglobinemia does not cause hemol-
ysis and the association of hemolytic anemia and sulfhemoglobinemia is in-
frequent, the latter will rarely require treatment, except for removing its
cause.

**Summary**

Sulfhemoglobinemia associated with Heinz body formation and acute
hemolytic anemia following contact with a fungicide, zinc ethylene bisdithio-
carbamate, is described in a Persian Jew whose red blood cells had low glu-
cose-6-phosphate dehydrogenase activity with low and unstable reduced
 glutathione and low catalase activity.

The fungicide, similarly to acetylphenylhydrazine, was capable of decreasing
in vitro the reduced glutathione of the patient's red blood cells, as well
as of those of other subjects with the same enzymatic defect.

The sulfhemoglobinemia and the hemolytic anemia are considered to have
been produced independently by the fungicide, the glucose-6-phosphate de-
hydrogenase deficiency having played a role only in the latter. The possibility
that the hypocatalasemia was a factor in rendering the patient's red blood
cells sensitive to the hemolysis- and sulfhemoglobin-producing action of the
fungicide is discussed.

The importance of zinc ethylene bisdithiocarbamate as a sulfhemoglobin-
producing and hemolytic agent is stressed, in view of the widespread use
of this fungicide.

**Summario in Interlingua**

Es describite un caso de sulfhemoglobinemia associate con le formation de
corpores de Heinz e acute anemia hemolytic post contacto con le fungicida
bisdithiocarbamato ethylenic de zinc. Le patiente esseva un judeo perse in
qui le erythrocytos habeva basse nivellos de activitate de dishydrogenase
de glucosa-6-phosphato con basse e instabile nivellos de reducite glutathiona e
basse activitate de catalase.

Le fungicida, simile in isto a acetylphenylhydrazina, esseva capace a
diminuer in vitro le reducite glutathiona del erythrocytos del patiente e etiam
de illos de alte subjectos con le mesme defecto enzymatic.

Es opinate que le sulfhemoglobinemia e le anemia hemolytic esseva
produce independenteemente per le fungicida e que le carentia de dishydro-
genase de glucosa-6-phosphato habeva un rolo solmente in le causation del
anemia. Le possibilitate que le hypocatalasemia esseva un factor in render
le erythrocytos del paciente sensibile al mecanismo per le qual le fungicida produceva hemolyse e sulfhemoglobina es discutite.

Viste le extense uso de bisdithiocarbamato ethylenic de zinc como fungicida, su capacitate de producir sulfhemoglobinemia e hemolyse es sublineate.

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REFERENCES

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Sulphemoglobinemia and Acute Hemolytic Anemia with Heinz Bodies Following Contact with a Fungicide—Zinc Ethylene Bis(dithiocarbamate)—in a Subject with Glucose-6-Phosphate Dehydrogenase Deficiency and Hypocatalasemia

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