Effect of Trisodium Calcium Diethylenetriaminepentaacetate on Bivalent Cations in Thalassemia Major

By Ursula Muller-Eberhard, Marion E. Erlandson, H. Earl Ginn and Carl H. Smith

Thalassemia major is one of the conditions in which severe secondary hemochromatosis occurs as a principal complication. This type of hemochromatosis is manifest at an earlier age than is primary hemochromatosis. In addition to parenteral administration of iron in the form of frequent blood transfusions, increased intestinal absorption permits marked accumulation of iron in patients with this disorder. Excess deposition of iron in tissues occurs in association with aberration in physiologic function of vital organs and with increased morbidity and early mortality in patients with thalassemia major. Consequently, prevention of extensive accumulation or elimination of excessive iron from the body might be an important factor in altering the course of the disease.

Recently Fahey et al. reported an appreciable urinary excretion of iron from patients with primary and secondary hemochromatosis following intravenous or intramuscular administration of a chelating agent, trisodium calcium diethylenetriaminepentaacetate (DTPA). The present study was designed to evaluate the effect of DTPA on excretion of iron in a group of patients with thalassemia major. The purpose of this presentation is to confirm the efficacy of this agent in removing iron and to comment upon changes in blood levels and urinary excretion of other bivalent cations following DTPA administration.

Patients

Seven patients, aged 3–25 years, with thalassemia major were studied. With the exception of the two youngest (M. C. and D. G.), all patients had been splenectomized 4 months to 18 years prior to this investigation. Histologic sections of liver and spleen tissues obtained at the time of splenectomy had demonstrated marked deposition of hemosiderin and varying degrees of fibrosis.

In the three oldest patients (T. P., R. C. and A. D.) evidence of impaired cardiac conduction was present and two of these (R. C. and A. D.) were being maintained on digoxin. Patient A. D. received intermittent diuretic therapy. However, no diuretic agent was used 3 days before or following injection of DTPA.

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*Geigy Laboratories.
Fig. 1.—Average iron excretion per 24 hours in seven patients with thalassemia major (2–5 determinations per patient). Normal excretion: up to 0.5 mg./24 hours. Individual grey bars represent consecutive 24-hour periods, the first starting at the time of drug administration.

**MATERIAL AND METHODS**

DTPA was administered 2 to 11 times over periods of 2 to 9 months by intramuscular injection, each individual dose containing 20–25 mg./Kg. The injections were rather painful. Therefore, DTPA was usually administered with a 1–2 per cent procaine solution. Twenty-four-hour collections of urine were obtained one or more times prior to injection of the drug and over one to three consecutive 24-hour periods beginning at the time of drug administration.

**Iron Determination**

Amounts of iron in serum and urine were measured by a slight modification of the method of Peters et al. Determinations were performed on centrifuged specimens of urine within 3 days of collection. Two blanks were used for each determination in order to correct for urinary color and reagents. All samples were handled and tested in iron-free equipment. Recovery of iron from urine containing 15 mg. DTPA per 100 ml. urine corresponded well with expected values.

**Copper, Magnesium and Calcium Determinations**

Serum and urinary copper determinations were performed at the Rockefeller Institute by courtesy of Dr. Alexander G. Bearn, and by means of a slight modification of the technic of Eden and Green. Additional serum and urine samples were dried at 90–100 C., ashed at 550 C., and reconstituted to the initial volume. Magnesium was then determined by the method of Simonsen, Westover and Wertman and calcium by the technic of Bachra, Dover and Sobel.10

**RESULTS**

1. **Urinary Excretion of Iron, Copper, Magnesium and Calcium**

The average urinary excretion of iron prior to and 1 to 3 days following administration of DTPA is illustrated in figure 1. Values for iron in speci-
mens collected before injection with DTPA were normal in the younger patients (0.03-0.34 mg./24 hours), but slightly above the normal maximum in the three older patients (0.53-2.34 mg./24 hours). The latter finding has also been reported in patients with primary and secondary hemochromatosis by other authors. The increased excretion of iron was limited to the period 12-24 hours after injection and was maximal within 6 hours. The least excretion of iron following medication was noted in the two youngest patients (1.5 and 2 mg./24 hours). Patient R. C., aged 24 years, eliminated the largest amount of iron, 40.18 mg./24 hours on one occasion.

Although greatest increases were seen in excretion of iron, changes were also noted for copper, magnesium and calcium.

The initial copper values obtained before administration of DTPA were greater than normal only in the two oldest patients, aged 19 and 24 years, (90.0-153.7 μg./hours) (fig. 2). The amount of copper in the urine was increased 1½-3½ times following DTPA injection and exceeded normal values in each instance. The increased copper excretion lasted only 1 day.

All values for magnesium excretion were less than normal or 0.75-3.84 mEq./24 hours (fig. 3) (normal, 5-8 mEq./24 hours). Following injection of DTPA the excretion of magnesium was increased 15-60 per cent above average initial values but still remained below the normal range. However, the enhancement of magnesium excretion lasted 2 or even 3 days in contrast to the duration of increase for iron and copper.

Excretion of calcium prior to DTPA injection was less than normal in five
Fig. 3.—Urinary magnesium excretion per 24 hours in six patients with thalassemia major. Normal excretion: 5–8 mEq. 24 hours. Individual grey bars represent consecutive 24-hour periods, the first starting at the time of drug administration.

Fig. 4.—Urinary calcium excretion per 24 hours in six patients with thalassemia major. Normal excretion: 2.5–8.75 mEq. 24 hours. Individual grey bars represent consecutive 24-hour periods, the first starting at the time of drug administration.
of six patients (0.08-1.20 mEq./24 hours) and increased 30 to 550 per cent above average initial values following medication\textsuperscript{11} (fig. 4). In the sixth patient, T. P., amounts of calcium in pretreatment urine specimens were 3.5-6.25 mEq./24 hours or well within the normal range while magnesium values determined in the same specimens were extremely low (0.75-1.33 mEq./24 hours). In normal subjects the range of 24-hour urinary calcium excretion is 2.5 to 8.75 mEq. on intakes of 10-30 mEq. of calcium per day. The approximated dietary intake of these patients was in the same range.\textsuperscript{11}

2. Serum Levels of Iron and Copper and Plasma Levels of Magnesium and Calcium

The serum iron values were high and the iron-binding protein saturated in all patients as has been previously reported for patients with thalassemia major.\textsuperscript{12} After the injection of 10 mg./Kg. of DTPA to one patient, serum samples were obtained 6 times at 30-minute intervals and then every 60 minutes for 6 hours. During this period no significant fluctuation in serum iron concentration was encountered.

Plasma calcium levels were low normal or slightly depressed before (4.36-4.68 mEq./100 ml.) and remained essentially unchanged after injection of DTPA. Serum copper levels were above normal before and after injection (119.5-189.0 \( \mu g./100 \text{ ml.} \)) (table 1). One patient's serum (R. C.) was tested before (168.6 \( \mu g./100 \text{ ml.} \)) and 6 hours post-injection (20 mg./Kg. DTPA) when the copper level was unchanged (174.8 \( \mu g./100 \text{ ml.} \)).

By contrast, plasma concentrations of magnesium tended to be very low in this series of patients with thalassemia major (0.67-1.69 mEq./L.) (table 1) (normal, 1.70 \( \pm \) 0.15). The two patients (and one additional infant) studied before drug administration showed significantly low initial values (1.20-1.53 mEq./L.). After injection of DTPA, further transient reduction was noted at 24-48 hours in most instances. However, fluctuation of magnesium levels were great in the two youngest children as well as in the infant who never received DTPA.

A possible adverse effect of DTPA on magnesium balance was observed in one patient, A. D., who after administration of 11 Gm. of DTPA over an 8-week period, developed progression of cardiac arrhythmias and complained of extreme weakness and muscular irritability. The plasma magnesium concentration was 0.86 mEq./L. and 6 days later, 0.67 mEq./L. The calcium level at the time of the second magnesium determination was 3.4 mEq./100 ml. Diuretic agents had also been administered. After magnesium and calcium supplements were given, the plasma levels of both cations rose and the patient improved clinically. He was 25 years old, the oldest of the group, and continued to have difficulties with cardiac failure shortly thereafter. No further DTPA injections were given.

**Discussion**

DTPA has a stronger affinity for iron than any other chelating agent evaluated in this respect. A nearly quantitative excretion in urine of radio-
Table 1.—Plasma Magnesium and Calcium and Serum Copper in Eight Patients with Thalassemia Major

<table>
<thead>
<tr>
<th>Years</th>
<th>Patients</th>
<th>Mg. (mEq./L.) before*</th>
<th>Ca (mEq./100 mL.) before*</th>
<th>Cu (μg./100 mL.) before*</th>
<th>Total Dose of DTPA</th>
<th>Time from Last Injection</th>
</tr>
</thead>
<tbody>
<tr>
<td>normal</td>
<td></td>
<td>1.70 ± 0.15</td>
<td>5.0 ± 0.5</td>
<td>108 ± 9.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8 12</td>
<td>D. C.</td>
<td>1.49</td>
<td>4.36</td>
<td></td>
<td>0.38 Gm.</td>
<td>2½ months</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.20</td>
<td>4.52</td>
<td></td>
<td>0.75 Gm.</td>
<td>3 months</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.53</td>
<td>—</td>
<td></td>
<td>—</td>
<td>4½ months</td>
</tr>
<tr>
<td>3</td>
<td>M. C.</td>
<td>1.26</td>
<td>3.47</td>
<td>119.5</td>
<td>0.70 Gm.</td>
<td>3 months</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.31</td>
<td>4.41</td>
<td></td>
<td>1.05 Gm.</td>
<td>6 days</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.70</td>
<td>4.67</td>
<td></td>
<td>—</td>
<td>3½ months</td>
</tr>
<tr>
<td>4 6  12</td>
<td>D. G.</td>
<td>1.48</td>
<td>4.35</td>
<td>151–179</td>
<td>3.0 Gm.</td>
<td>5 months</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.11</td>
<td>4.32</td>
<td></td>
<td>3.5 Gm.</td>
<td>2 weeks</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.64</td>
<td>—</td>
<td>189.0</td>
<td>—</td>
<td>1 day</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.95</td>
<td>—</td>
<td></td>
<td>4.0 Gm.</td>
<td>—</td>
</tr>
<tr>
<td>7</td>
<td>S. I.</td>
<td>1.55</td>
<td>4.82</td>
<td>151–179</td>
<td>3.0 Gm.</td>
<td>3½ weeks</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.51</td>
<td>4.78</td>
<td></td>
<td>3.5 Gm.</td>
<td>2 weeks</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.34</td>
<td>4.69</td>
<td></td>
<td>4.0 Gm.</td>
<td>1 day</td>
</tr>
<tr>
<td>11</td>
<td>S. L.</td>
<td>1.33</td>
<td>4.68</td>
<td>185.5</td>
<td>0.68 Gm.</td>
<td>1 day</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.98</td>
<td>4.78</td>
<td></td>
<td>1.35 Gm.</td>
<td>4 months</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.18</td>
<td>4.03</td>
<td></td>
<td>—</td>
<td>4½ months</td>
</tr>
<tr>
<td>19</td>
<td>T. P.</td>
<td>1.27</td>
<td>4.51</td>
<td></td>
<td>2.25 Gm.</td>
<td>2 days</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.40</td>
<td>—</td>
<td></td>
<td>5 weeks</td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>R. C.</td>
<td>1.69</td>
<td>3.90</td>
<td>168.6</td>
<td>3.62 Gm.</td>
<td>40 hours</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.54</td>
<td>3.86</td>
<td></td>
<td>6.62 Gm.</td>
<td>3 weeks—6 hrs.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>—</td>
<td></td>
<td></td>
<td>—</td>
<td>post 3rd inj. that week</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.87</td>
<td>3.28</td>
<td></td>
<td>7.62 Gm.</td>
<td>2 days</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.14</td>
<td>3.78</td>
<td></td>
<td>3 weeks</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.05</td>
<td>—</td>
<td></td>
<td>3 weeks</td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>A. D.</td>
<td>0.86</td>
<td>—</td>
<td>8.62 Gm.</td>
<td>2 days</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.67</td>
<td>3.40</td>
<td></td>
<td>1 week</td>
<td></td>
</tr>
</tbody>
</table>

*“Before” and “after” refer to time relation of test to injection of DTPA.
Results listed under “after” represent consecutive 24-hour periods, the first beginning at the time of drug injection.
iron bound to DTPA occurs after parenteral administration. Thus accurate determination of urinary iron would appear to be an adequate index of iron excretion induced by DTPA. Recovery data from experiments performed in this laboratory confirm the impression that iron excreted in combination with DTPA was accurately measured by the method employed.

This study was originally designed to investigate the long-term effect of DTPA on patients with thalassemia major. However, the occurrence of a state of probable calcium and magnesium deficiency in one of the patients during the course of therapy with DTPA prompted an investigation of possible changes of other cations in blood and urine during such treatment.

The unchanged plasma levels and the increased urinary excretion of calcium after the injection of DTPA are to be expected in view of the fact that this chelating agent was administered in the form of its calcium salt. The invariably low initial plasma calcium levels and urinary excretion values (table 1, fig. 4) must be related to the disease itself and may be related to the clinical finding of osteoporosis.

Serum copper values were above normal in all instances, a finding characteristic in patients with cirrhosis of the liver. Greater than normal urinary copper values are also found in cirrhosis. Copper excretion in the urine of patients with thalassemia major after administration of DTPA has not been studied previously. Copper excretion was markedly enhanced following administration of DTPA, being less so, however, than following either BAL or penicillamine.

The average amount of iron removed by DTPA was similar in the older patients in this series and in those adults reported by Fahey et al. The amounts found in both these series exceed those which have been measured following administration of EDTA. One Gm. of DTPA resulted in a maximal elimination of 40.18 mg. iron in a 24-hour period. Although this amount of iron is only approximately one-sixth of that removed by phlebotomy of 500 ml. of whole blood, multiple injections given over a long period of time appear to be a possible method of minimizing accumulation of iron.

Initial plasma concentrations of magnesium in patients with thalassemia major were found to be either low normal, or more often, significantly reduced. After injection of DTPA a further transient reduction of magnesium levels was usually observed. The explanation for the low initial magnesium levels in thalassemia major is at present obscure and may be related to the basic disease.

Increased urinary excretion of magnesium following DTPA administration was of interest particularly in view of its persistence for 3 days rather than just 24 hours after injection of drug. It is possible that reduction of plasma magnesium and increased urinary excretion of this cation may have resulted from some secondary effect rather than being directly related to chelation by DTPA.

**Summary**

The effect of a chelating agent, trisodium calcium diethylenetriaminepentaacetate (DTPA), in seven patients with thalassemia major and sec-
Secondary hemochromatosis is reported. Urinary excretion of iron, copper, magnesium, and calcium was measured prior to and following intramuscular injections of DTPA. As much as a 16-fold increase in excretion of iron (up to 40 mg./24 hours) and a 3½-fold increase in excretion of copper were achieved. Magnesium and calcium in urine remained consistently below the average range for normal individuals although excretion of magnesium increased 15-60 per cent and calcium as much as 550 per cent following administration of this agent.

While serum levels of copper were high and plasma calcium levels low normal or slightly reduced, plasma magnesium levels were found to be slightly to significantly low in all patients studied. The latter were transiently further reduced following injection of DTPA and may have been related to symptoms of magnesium deficiency in one patient.

This agent was effective in removal of iron particularly in the older patients with secondary hemochromatosis. However, attention is drawn to the possible hazardous effect of DTPA on magnesium metabolism in patients with thalassemia major.

**SUMMARIO IN INTERLINGUA**

Le effecto de un agente de chelation, diethylenetriaminopentaacetato trisodic calcic (DTPA), in septe patientes con thalassemia major e hemochromatosis secundari es reportate. Le excretion urinari de ferro, cupro, magnesium, e calcium esseva mesurate ante e post le injection intramuscular de DTPA. Augmentos del excretion de ferro per un factor de usque a dece-sex (atingente 40 mg per 24 horas) e del excretion de cupro per un factor deusque a tres e medie esseva obtenite. Le magnesium e le calcium in le urina remaneva uniformemente infra le ordine medie pro subjectos normal, ben que le excretion de magnesium cresceva par 15 a 60 pro cento e illo del calcium per usque a 550 pro cento post le administration del agente mentionate.

Durante que le nivellos seral de cupro esseva alte e le nivellos plasmal de calcium basse, normal, o levemente reducite, le nivellos plasmal de magnesium esseva leve- o significativamente basse in omne le patientes studiate. Iste ultime nivellos esseva transientemente reducite ancora plus post le injection de DTPA e esseva possibilmente relationate con le symptomas de carentia de magnesium in un caso.

Le agente esseva efficace in le elimination de ferro particularmente in patientes de etate plus tosto aviantate con hemochromatosis secundari. Tamen, es signalate le possibile effecto hasardose de DTPA super le metabolismo de magnesium in patientes con thalassemia major.

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