Effect of Ascorbic Acid in Vivo on Labeling of Red Cells by Radioactive Sodium Chromate

By J. William Poppell, Margaret E. Hood and Jerome J. Decosse

Since the initial work of Gray and Sterling, the determination of red cell mass with radioactive sodium chromate has undergone several modifications. Read developed the technic of adding ascorbic acid in vitro to the blood-sodium chromate mixture to reduce chromate to trivalent chromium and prevent further uptake by red cells. The inhibition of chromium uptake by ascorbic acid supported the concept of Gray that a reducing agent prevents complete tagging of erythrocytes by chromate in vivo.

In 1956, Cooper et al. suggested that radioactive sodium chromate could be mixed directly with blood in acid citrate dextrose (ACD), and that this mixture led to a higher degree of red cell labeling than when either heparin or sodium citrate alone was used. Since ACD solution did not materially reduce the chromate ion within the first few hours of mixing, these authors added sodium chromate to the ACD solution prior to addition of whole blood. In 1957, Cunningham et al. stated that ACD solution does reduce chromate and that such mixtures could not be used effectively longer than 4 hours after mixing. By tracer technics and spectrophotometric analysis of mixtures of ACD and \( \text{K}_2\text{Cr}_5\text{O}_4 \), these authors documented reduction of chromate by acid citrate dextrose formation to a chromic-citrate complex.

In the course of doing clinical blood volumes we found on several occasions that the per cent of red cells labeled in vitro was decreased markedly from the approximate 90 per cent expected. Further investigation indicated that in vivo concentrations of ascorbic acid affected the labeling of human red cells in vitro.

Methods

Clinical

Fifty-two blood samples were taken from 17 surgical patients for determination of whole blood ascorbic acid concentration and per cent tagging with radioactive sodium chromate. Of the 17 surgical patients selected, eight had no previous known ascorbic acid therapy (table 1). The remaining nine patients received ascorbic acid orally for 3 to 6 days prior to the test referred to below as ascorbic acid loading. The daily dose ranged from 100 to 1000 mg. None of the patients had alterations in their diet, or unusual losses through diarrhea or chronic gastrointestinal tract disease.

In each instance, 20 cu. cm. of heparinized blood were withdrawn for control determination of \( \text{Cr}^{51} \) labeling and blood ascorbic acid concentration. An infusion of 500 cc. 5 per cent dextrose in water containing from 100 to 1000 mg. of ascorbic acid was started...
### Table 1

<table>
<thead>
<tr>
<th>No. Patients</th>
<th>Previous Vitamin C Therapy and Test Dose</th>
<th>Initial</th>
<th>End of Test</th>
<th>1 Hour Later</th>
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</thead>
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<tr>
<td></td>
<td></td>
<td>% Tag Cr&lt;sup&gt;81&lt;/sup&gt;</td>
<td>Vitamin C (mg./100 cc.)</td>
<td>% Tag Cr&lt;sup&gt;81&lt;/sup&gt;</td>
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<td>4</td>
<td>No previous vitamin C therapy test—100 mg. i.v.</td>
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<td></td>
<td>average</td>
<td>93.8</td>
<td>0.43</td>
<td>92.7</td>
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<td></td>
<td>range</td>
<td>92.6–95.3</td>
<td>.35–.51</td>
<td>92.1–94.0</td>
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<td>2</td>
<td>No previous vitamin C therapy test—500 mg. i.v.</td>
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<tr>
<td></td>
<td>average</td>
<td>91.7</td>
<td>0.77</td>
<td>87.4</td>
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<tr>
<td></td>
<td>range</td>
<td>89.6–93.8</td>
<td>.55–.98</td>
<td>86.2–88.5</td>
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<tr>
<td>2</td>
<td>No previous vitamin C therapy test—1000 mg. i.v.</td>
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<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>average</td>
<td>92.0</td>
<td>0.87</td>
<td>83.2</td>
</tr>
<tr>
<td></td>
<td>range</td>
<td>91.3–92.7</td>
<td>.85–.89</td>
<td>78.4–88.0</td>
</tr>
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<td></td>
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<tr>
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<td>average</td>
<td>83.3</td>
<td>1.36</td>
<td>78.8</td>
</tr>
<tr>
<td></td>
<td>range</td>
<td>76.4–88.7</td>
<td>.66–1.85</td>
<td>71.5–86.2</td>
</tr>
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<td>Received 1000 mg. vitamin C/day for 5 days test—100 mg. i.v.</td>
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<tr>
<td></td>
<td>80.4</td>
<td>1.35</td>
<td>79.5</td>
<td>1.32</td>
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<tr>
<td>1</td>
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</tr>
<tr>
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<td>91.6</td>
<td>1.58</td>
<td>86.7</td>
<td>2.30</td>
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<tr>
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<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>87.6</td>
<td>1.09</td>
<td>87.6</td>
<td>1.00</td>
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</table>

The ascorbic acid concentrations in whole blood and the per cent tag with Cr<sup>81</sup> are segregated into the kind of pretest ascorbic acid loading and the method of ascorbic acid administration during the test.
intravenously. At the end of the infusion, usually in 1 hour, a second blood sample was withdrawn. A third blood specimen was taken 1 hour later. The samples were collected from a site distant from the infusion in syringes wetted with heparin.

Technic of Sodium Chromate (Na$_2$Cr$^{51}$O$_4$) Labeling

The 20 cc. blood samples were placed in Squibb "Saftag" vials containing 10 cc. of ACD solution (dextrose, anhydrous, 125 mg.; sodium citrate, 250 mg.; and citric acid, 80 mg.). Sterility was not maintained. Squibb Chromitope, specific activity 190 $\mu$c./mg., was then added to the blood-ACD mixture in quantities ranging from 1.4 $\mu$c. to 10 $\mu$c. in volumes of 0.05 cc. to 0.2 cc. The bottle was swirled occasionally as it remained at room temperature for 30 minutes. At the end of this interval a 5 cc. aliquot was removed for determination of the per cent tag. A hematocrit was determined on a portion of this aliquot using a Van Allen hematocrit tube spun at 3000 rpm for 30 minutes. One ml. of whole blood and 1 ml. of supernatant were each counted in a well type scintillation counter to an accuracy of within 1 per cent.$^6$ With this system the yield for Cr$^{51}$ averaged 79,000 cpm./$\mu$c./ml. over a background of 130 cpm. Per cent tag was then calculated using the following formula:

$$\text{Per cent Tag} = \frac{\text{cpm/cc. whole blood} - \left[\frac{(1-\text{Hct}) \text{ cpm/cc. supernatant}}{\text{cpm/cc. whole blood}}\right]}{\times 100}$$

Technic of Ascorbic Acid Determinations

The remaining 25 cc. of blood ACD mixture were exposed to an atmosphere of 100 per cent nitrogen. The vial was sealed and placed in wet ice until analyzed. Ascorbic acid concentration of whole blood was determined by the method of Roe and Kuether.$^7$ The per cent tag and the ascorbic acid levels were measured by different laboratories, with neither knowing the other's results. Value of ascorbic acid were corrected to in vivo concentrations on the basis of the known dilution caused by mixing with ACD solution.

RESULTS

Ascorbic Acid Studies

Ascorbic acid concentrations in whole blood and per cent of tagging by Cr$^{51}$ were determined in 52 blood samples from the 17 patients studied. A linear correlation was found between the in vivo blood ascorbic acid level and the per cent tag of the donors' blood (fig. 1). As ascorbic acid concentrations increased, the per cent tagging with Cr$^{51}$ decreased. This relationship with 95 per cent confidence limits (broken line) is graphed in figure 1. Calculation of the regression equation yields: $Y = 54.6 - 5.17X; r = 0.79$.

These data also revealed a clear difference both in per cent tag and ascorbic acid concentration of the control blood samples between those who had no ascorbic acid therapy and those who were loaded before the test dose (table 1). After intravenous ascorbic acid administration there was an increase in blood ascorbic acid concentration and a corresponding decrease in per cent tagging in both groups, proportionate to the amount of ascorbic acid administered.

An 89.6 per cent tagging was the lowest initial tagging found in any of the eight patients who did not have previous ascorbic acid therapy. The mean value in these patients was 92.8 per cent, ranging from 89.6 per cent to 95.3 per cent. In this group the ascorbic acid concentration in the initial whole blood sample averaged .62 mg./100 cc., ranging from .35 to .98 mg./100 cc.
Fig. 1.—Cr\(^{51}\) per cent tagging as a function of ascorbic acid blood levels, representing 52 studies in 17 patients. The broken lines are the 95 per cent confidence limits. \(Y = \text{per cent tag} \); \(X = \text{ascorbic acid level}\).

Five of the eight patients had values which were below the reported normal range (0.7–2.0 mg./100 cc\(^8,10\)).

In the nine patients who received ascorbic acid loading in varying amounts before the infusion, the tagging of the control blood sample averaged 84.5 per cent, ranging from 76.4 per cent to 91.6 per cent. These patients had ascorbic acid concentrations which averaged 1.35 mg./100 cc. and ranged from 0.65 to 1.85 mg./100 cc.

**Discussion**

Gray and Sterling\(^1\) concluded that chromate ion (anionic hexavalent state) diffuses through the red cell membrane to be bound by hemoglobin within the cell, probably after reduction therein to the chromic ion (cationic trivalent state). Seventy to 78 per cent of the bound chromium is found with the globin fraction.\(^3,11\)

Red cell tagging approximates 90 per cent under optimal circumstances.\(^1,3,12\)

The inability to achieve complete tagging has been credited to "plasmatic reducing substances"\(^4\) which, by reducing the ion and allowing binding to
plasma protein, prohibit its incorporation within the red cell. In vitro, red cell tagging may be modified by a number of conditions. The labeling power of sodium chromate is diminished by decreasing the hydrogen ion concentration of the solution,\(^5\) and by decreasing the hematocrit.\(^4\) The reaction is accelerated by exposure to light and it is a function of time\(^5\) and temperature.\(^4\) ACD solution provides a higher degree of labeling than heparin or sodium citrate.\(^4\) The labeling power of ACD solution is decreased by increasing the citrate concentration and, to a lesser extent, by increasing the dextrose concentration.\(^5\) These authors also documented a sharp reduction in labeling when the ACD solution was caramelized by autoclaving at 100°C for 4 hours.

Red cell labeling can be prevented by addition of ascorbic acid. When as little as 50 mg. of ascorbic acid are added to 500 µg. of radioactive sodium chromate, the red cell uptake of this tracer is prevented both in vitro and in vivo.\(^3\) Cooper\(^4\) noted that 8 or more mg. of ascorbic acid per 3 ml. of blood were able to block chromatol labeling almost completely, while the addition of only 1 mg. reduced tagging to 15 per cent. Presumably ascorbic acid reduces chromate to the chromic ion, which, as previously noted, is unable to become bound to erythrocytes.

Evidence is present here to indicate that the reducing action of physiologic concentrations of blood ascorbic acid in vivo can modify radioactive sodium chromate tagging of red cells, and that blood ascorbic acid concentrations have a significant role in the inability to tag 100 per cent of red cells with radioactive sodium chromate. The results indicate that a higher per cent tag may be obtained if oral and parenteral ascorbic acid therapy are discontinued before withdrawing blood to be used for red cell mass or survival studies.

The response of blood ascorbic acid levels to administration of intravenous ascorbic acid has been used as a measure of ascorbic acid deficiency.\(^8\) In this limited number of patients tested by varying amounts of intravenous ascorbic acid, the presence or absence of pre-test ascorbic acid “loading” did not affect the degree of change in vitamin C levels at the end of the infusion or 1 hour later, although five patients had baseline blood concentrations of ascorbic acid that were below the normal range.

**Summary**

Fifty-two whole blood samples from 17 patients were analyzed for ascorbic acid concentration and Cr\(^{51}\) tagging. Physiologic concentrations of ascorbic acid in whole blood in vivo reduced radioactive sodium chromate in vitro and impaired tagging.

**Summario in Interlingua**

Cinquanta-duo specimens de sanguine total, obtenite ab 17 patientes, esseva analysate pro le concentration de acido ascorbic e le marcation de erythrocyes con Cr \(^{51}\). Esseva trovate que concentrationes physiologic de acido ascorbic in sanguine total in vivo reduceva le radioactive chromato de natrium in vitro e disturbava le marcation.
ACKNOWLEDGMENT

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REFERENCES


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