Allopurinol and Iron Metabolism in Man

By John D. Boyett, William R. Vogler, Valdir de Paula Furtado and Frederick H. Schmidt

The enzyme, xanthine oxidase, is thought to play a central role in the mobilization of storage iron from the liver. In the presence of xanthine oxidase, xanthine is oxidized to uric acid, the enzyme acting as electron acceptor in the oxidation reaction. Coupled to the oxidation of uric acid is the reduction of ferric ferritin to the ferrous state. The livers of immature rats contain very little xanthine-oxidase activity, and the hepatic ferritin content is greater than that found in normal adult rats. As these animals approach maturity, xanthine-oxidase activity rapidly rises to the adult level, and there is a parallel fall in ferritin to normal adult levels. In the adult rat, xanthine oxidase is present in excess, such that the factor limiting the rate of release of ferritin iron is the cellular concentration of xanthine and hypoxanthine.

In the rare disorder, xanthinuria, there is a gross deficiency of xanthine oxidase. One such patient, a 47 year old male, also has hemochromatosis. Mazur and Sackler have presented evidence that xanthine-oxidase activity is low in hepatic tissues in hemochromatosis and cirrhosis. Excessive hepatic iron deposits in cirrhosis may be the result of acute and chronic deficiencies of protein. The same is true in patients with kwashiorkor, and, in addition, hepatic xanthine-oxidase activity is low and returns to normal levels following administration of diet containing adequate amounts of protein. Xanthine-oxidase activity is low in cirrhotic livers and is associated with extensive hepatic iron deposits.

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The ferritin-apoferritin system is thought to play a role in iron absorption. Granick has proposed that ferritin is a vehicle for iron absorption and a participant in the "mucosal block." Crosby has challenged this concept and has suggested that ferritin in villous epithelial cells cannot be released and hence is lost from the body when the cell is sloughed. If reduced xanthine-oxidase activity results in ferritin accumulation, iron absorption might be increased, decreased, or unaltered. Accumulation of ferritin in villous epithelial cells which are to be sloughed would reduce iron absorption. If the ferritin-apoferritin system is part of the prime mover of iron across the mucosal surface, iron absorption might be enhanced. An intermediate effect might result in no net change.

Allopurinol (4-hydroxypyrazolo (3,4d) pyrimidine) is a potent inhibitor of xanthine oxidase. Administration of this agent results in prompt lowering of serum uric acid concentration, decreased urinary uric acid excretion, and reciprocal rise in urinary oxypurine excretion. Extensive clinical studies have shown that this drug is quite effective in the treatment of various types of hyperuricemia, and significant side effects have not attended its use. Powell and Emmerson fed allopurinol to rats and noted significant increase in hepatic storage iron. Davis and Deller studied iron absorption in 21 healthy volunteers before and after administration of allopurinol. Iron was used as a tracer, and a whole-body counter was used to measure iron absorption. These investigators found no evidence that iron absorption was influenced by allopurinol therapy. Rundles and associates measured the absorption of radioiron in a patient with polycythemia and iron deficiency who was receiving allopurinol therapy. They noted no change. However, they noted a serum iron of 197 μg per cent in an additional patient on long-term allopurinol therapy. Powell and Emmerson mention a single patient whose serum iron rose from a base line level of 154 to 270 μg per cent after seven months continuous therapy with allopurinol.

This is a report of the effect of allopurinol on radioiron absorption, storage, and red cell incorporation.

**Materials and Methods**

Nine patients with hyperuricemia were admitted to a metabolic ward and fed a diet containing 30 mg of purine nitrogen during the period of observation. One patient had hyperuricemia; two patients had hyperuricemia, acute gouty arthritis, and rheumatoid arthritis; and six patients had chronic tophaceous gout with histories of recurrent gouty arthritis.

In order to assess adequacy of iron stores and presence of hepatic or renal disease, the following laboratory data were obtained on all patients: complete blood counts, serum uric acid, serum iron and total iron-binding capacity, serum glutamic oxaloacetic transaminase, serum alkaline phosphatase, total serum protein and serum electrophoresis, serum creatinine, stools for occult blood, and bone marrow aspiration with biopsy and iron stain.

Each patient received two doses of ferrous radioiron as described below. In each case oral or intravenous ferrokinetics were assessed with and without administration of allopurinol. In four oral studies, drug dosage sequences were reversed as indicated in order to minimize the known effect of phlebotomy upon iron absorption.

Allopurinol was kindly supplied by Burroughs Wellcome & Co., Tuckahoe, New York.
Oral Studies
Two patients were given 35 to 40 μc. of $^{59}$Fe citrate orally without carrier. All patients were in the fasting state at the time of isotope administration. Prior to isotope administration, plasma volume was determined with Evans blue. Blood samples were collected daily for determination of plasma and whole blood radioactivity and hematocrit. On the eighth day of observation, allopurinol was instituted in a dose of 300 mg. every 12 hr. and increased to 600 mg. every 12 hr. for six doses 48 hours prior to the second dose of isotope. The original drug schedule was then resumed and continued throughout the period of observation.

Four patients were given allopurinol 300 mg. every 12 hr. for 4 days. Dosage was then increased to 600 mg. every 12 hr. for a total of six doses, and the original dose schedule was resumed. The drug was administered for a total of ten days. Two days after the dose of allopurinol was increased to 600 mg. every 12 hours, $^{59}$Fe citrate, 35 to 40 μc., was given orally, in the fasting state, without carrier or ascorbic acid. Immediately prior to the administration of the isotope, plasma volume was determined with Evans blue. Ten days after cessation of allopurinol therapy, a similar dose of $^{59}$Fe citrate was given orally and plasma volume was again determined with Evans blue. Blood samples were obtained daily for determination of whole blood and plasma radioactivity and hematocrit.

All stools were collected during the period of observation of the patients on the oral study. Those from days 1 through 14 and days 14 through 28 of study were pooled, weighed, and homogenized. An aliquot was taken from each homogenate, weighed, and counted and from this was calculated total fecal radioactivity.

Intravenous Studies
Under sterile conditions, 7 to 10 ml. of plasma from the patient were incubated at 37 C. for 15 min. with $^{59}$Fe ferrous citrate. Approximately 7 μc. of radioiron were injected. At the same time, plasma volume was measured with Evans blue. Plasma clearances and red cell uptake were measured as previously described.20 Nine to 12 days after the initial study, allopurinol, in a dose of 300 mg. every 12 hr. was begun and continued throughout the second ferrokinetic study which began 14 days after the first.

Daily organ counts over spleen, liver, and sacrum were measured according to the method of Pollycove and Mortimer.20

All radioactive samples were counted in a Nuclear Chicago Automatic Gamma Sample Changer, Model C-120, with 1 in. sodium iodide crystal. Each sample was counted for a total of 10,000 counts. Organ counts were measured with a 2 in. sodium iodide crystal mounted in a Nuclear Chicago DS-5 probe and coupled to a Nuclear Chicago Model 132 scaler. The latter counts were made to exceed 10 times background.

Erythrocyte Radioiron Uptake Model
For purposes of data reduction, it was assumed that the behavior of $^{59}$Fe could be approximated as a three-compartment catenary, described in equation 1.

$$K_1 \quad K_2 \quad C$$

At t=0, A=A₀, B₀=0, C₀=0

The C compartment represents erythrocyte radioactivity and

$$C = A_0 \left( \frac{K_2}{K_1 - K_2 e^{-K_1 t}} + \frac{K_2}{K_2 - K_1 e^{-K_2 t}} \right) + 1$$

$A_0$, $K_1$, $K_2$, and background radioactivity were estimated on the IBM Model 1410 digital computer using the Marquardt nonlinear regression program.22 This program, in addition to estimates of parameters, gives estimates of the standard errors of the parameters.
Table 1.—Effect of Allopurinol on Erythrocyte Incorporation and Fecal Excretion of Radioiron Given Orally

<table>
<thead>
<tr>
<th>PT #</th>
<th>Study #</th>
<th>Drug Schedule</th>
<th>Asymptote* CPM/ML RBC</th>
<th>Std. Error CPM/ML RBC</th>
<th>Erythrocyte† 59Fe Uptake % (Days)</th>
<th>Significant Difference</th>
<th>Fecal Excretion % Total Dose 59Fe</th>
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<tbody>
<tr>
<td>1</td>
<td>2</td>
<td>Control</td>
<td>2243</td>
<td>786</td>
<td>20.9 (9)†</td>
<td>No</td>
<td>47</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>Allopurinol</td>
<td>5221</td>
<td>213</td>
<td>36.3 (9)</td>
<td>No</td>
<td>15</td>
</tr>
<tr>
<td>1</td>
<td>2</td>
<td>Control</td>
<td>574</td>
<td>146</td>
<td>4.4 (14)</td>
<td>No</td>
<td>82</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>Allopurinol</td>
<td>1433</td>
<td>7914</td>
<td>13.1 (14)</td>
<td>No</td>
<td>28</td>
</tr>
<tr>
<td>3</td>
<td>2</td>
<td>Allopurinol</td>
<td>6934</td>
<td>46</td>
<td>16.5 (14)</td>
<td>Yes</td>
<td>31</td>
</tr>
<tr>
<td>4</td>
<td>2</td>
<td>Control</td>
<td>5122</td>
<td>76</td>
<td>68.1 (14)</td>
<td>Yes</td>
<td>6</td>
</tr>
<tr>
<td>5</td>
<td>2</td>
<td>Control</td>
<td>6197</td>
<td>2894</td>
<td>45.6 (14)</td>
<td>No</td>
<td>36</td>
</tr>
<tr>
<td>6</td>
<td>2</td>
<td>Allopurinol</td>
<td>3954</td>
<td>174</td>
<td>41.1 (14)</td>
<td>No</td>
<td>40</td>
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<tr>
<td>6</td>
<td>2</td>
<td>Control</td>
<td>5464</td>
<td>162</td>
<td>42.9 (14)</td>
<td>No</td>
<td>79</td>
</tr>
<tr>
<td>6</td>
<td>2</td>
<td>Allopurinol</td>
<td>7320</td>
<td>862</td>
<td>27.4 (14)</td>
<td>No</td>
<td>38</td>
</tr>
<tr>
<td>6</td>
<td>2</td>
<td>Control</td>
<td>5156</td>
<td>476</td>
<td>48.1 (14)</td>
<td>No</td>
<td>34</td>
</tr>
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</table>

*Radioactivity, CPM/mL. RBC, predicted from model at t = ∞.
†Uptake of 59Fe by erythrocyte mass, per cent total dose. Number of days following dose indicated in parentheses.
§Second study terminated on ninth day. Thirteen day uptake on 1st study 27.6 per cent.

Plasma Clearance Model

3. \[ Y = B_1 + B_2 e^{-B_3 t} + B_4 e^{-B_5 t} + E \]
   \[ Y = \text{Plasma } 59\text{Fe activity in CPM/mL uncorrected for background} \]
   \[ B_1 = \text{Estimate of background count rate} \]
   \[ B_2 = \{ \text{Intercepts at } t = 0 \} \]
   \[ B_3 = \{ \text{Biologic decay constants} \} \]
   \[ B_5 = \{ \text{Error} \} \]

For measurement of plasma clearance of 59Fe (compartment A), equation 3 was used. Parameters (B's) were estimated by nonlinear regression analysis on a digital computer. An F test was used to test the null hypothesis that \( B_1 \) (control) = \( B_1 \) (treatment). All radioactive samples were assayed by recording the time for 10,000 counts, yielding a constant counting error of 1 per cent. For this reason, background count was included in the data to be fitted, and a logarithmic transformation was performed on all data before regression to assure proper weighting.

RESULTS

The findings of this study are presented in Tables 1 and 2. Nonlinear analysis of the erythrocyte iron uptake failed to yield any significant difference in control or allopurinol phases of the study in 5 of 6 subjects. This was true of both the rate of uptake and the absolute amount expressed as total dose of radioactivity which appeared in the red cell mass 14 days after the tracer was given. A significant difference was present in one patient.

Fecal excretion of radioiron after oral dose tended to be lower when allopu-
### Table 2.—Estimates of Plasma Clearance Rates Using Nonlinear Regression Analysis on Digital Computer

<table>
<thead>
<tr>
<th>Pt</th>
<th>T/2 Min.</th>
<th>Control B_1</th>
<th>S.E.</th>
<th>B_2</th>
<th>S.E.</th>
<th>Allopurinol B_1</th>
<th>S.E.</th>
<th>B_2</th>
<th>S.E.</th>
<th>Significant Difference 5% Level</th>
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<tr>
<td>7</td>
<td>64</td>
<td>-0.010801</td>
<td>0.0016178</td>
<td>—</td>
<td>—</td>
<td>-0.0082996</td>
<td>0.001508</td>
<td>—</td>
<td>—</td>
<td>yes</td>
</tr>
<tr>
<td>8</td>
<td>77</td>
<td>-0.00897348</td>
<td>0.00137878</td>
<td>—</td>
<td>—</td>
<td>-0.0077694</td>
<td>0.0011471</td>
<td>—</td>
<td>—</td>
<td>yes</td>
</tr>
<tr>
<td>9</td>
<td>83</td>
<td>-0.00833186</td>
<td>0.00069085</td>
<td>-0.0018203</td>
<td>0.0014969</td>
<td>0.00119868</td>
<td>0.002706</td>
<td>0.0062402</td>
<td>0.0034507</td>
<td>yes, no</td>
</tr>
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</table>
Fig. 1.—Distribution of radioiron following an intravenous dose before and after allopurinol. Per cent uptake over organs was calculated as that fraction of the total counts (liver, spleen, and sacrum) measured over the specified organ on each day. RBC per cent uptake calculated as fraction of total counts injected using Evans blue plasma volume and hematocrit to determine total red cell mass. Plots are the average percentages of three determinations.

Allopurinol was being given (Table 1). Mean fecal excretion for the allopurinol and control phases was 26.3 and 49.8 per cent, respectively. However, this difference is not statistically significant. Further, it will be noted that the total of fecal excretion and red cell uptake did not approach 100 per cent in most patients.

In all 3 patients given an intravenous dose of $^{59}$iron, there was a highly significant difference between the rates of clearance of the tracer from the plasma during the 2 phases of study. In 2 of the 3 patients, clearance was prolonged during allopurinol therapy. The reverse was true in the other patient (Table 2). Even so, the rates of clearance remained within normal limits.
In those receiving intravenous radioiron, there was no detectable difference between the organ counts during the drug and control phases. The percentage distribution of the total counts over the spleen, liver, and marrow were averaged for each day and plotted in Figure 1 for the control and treatment period. Also shown in Figure 1 are the averages of the per cent red cell uptake following the intravenous dose. These were similar in the control and allopurinol period.

**Comments**

In a preliminary report, we tentatively suggested that allopurinol might modestly affect iron absorption or iron metabolism. The addition of 2 more patients to our series and statistical testing of the data do not support this initial impression.

In one of our patients (No. 3), a significant increase in red cell iron uptake occurred during the second phase of study. It will be noted that this was the control phase (Table 1). His serum iron concentration and per cent saturation of transferrin were low normal. Iron stain of Vim-Silverman marrow biopsy did not reveal stainable iron. This patient has sero-positive rheumatoid arthritis, psoriasis and chronic tophaceous gout, and a past history of gastrointestinal bleeding thought to be due to salicylate-induced gastritis. Although he had received iron therapy for presumed iron deficiency, it was concluded that his iron stores were marginal on admission and that the blood-letting during phase I (approximately 150 ml.) had rendered him slightly iron deficient, resulting in enhanced absorption of iron.

Although Powell and Emmerson noted hepatic siderosis in rats fed allopurinol, the general impression found in the literature is that the drug produces little alteration in iron absorption or metabolism. Our data are consistent with this latter view. As mentioned above, the total fecal excretion and red cell uptake did not approach 100 per cent in most of our subjects given oral radioiron. This was disappointing in that it did not allow us to draw more meaningful conclusions about iron absorption. This, however, is in keeping with common experience relative to the inadequacies of fecal radioiron studies. It is to be stressed that Davis and Deller, using sensitive whole-body counting technics, detected no evidence of alteration of iron absorption by allopurinol.

The rate of plasma radioiron clearance was significantly different in all 3 subjects studied in the drug and control phases. However, these values are all within normal limits and the differences may be ascribable to physiologic variations. Further, no differences were to be noted between organ radioactivity in the drug and control periods in any of the 3.

In contrast to the findings of Pollycove and Mortimer, we found that plasma clearance followed a single exponential decay in 2 patients and a double in one, rather than a 3 exponential decay equation in the Pollycove model. This would suggest that feedback from the labile storage pool in these patients was exceedingly small. Since all 3 patients were considered to be normal hematologically, the differences must be ascribed to technic or mathematical handling of the data.
Summary

Oral and intravenous ferrokinetic studies were carried out in 9 patients with gout or hyperuricemia to determine the effect of allopurinol on iron absorption, storage, and red cell incorporation. Two studies were carried out in each patient: one during the control period and the other while receiving allopurinol. Allopurinol had no measurable effect on iron metabolism.

Summary in Interlingua

Orale e intravenose studios ferrocinetic esseva effectuate in novem patientes con gutta o hyperuricemia pro determinar le effecto de allopurinol super le absorption, le thesaurisage, e le incorporation in erythrocytos de ferro. Duo studios esseva effectuate in cata-un del patientes, i.e., le prime durante le periodo de controlo e le secunde durante le administration de allopurinol. Allopurinol non exerceva un mensurabile effecto super le metabolismo de ferro.

Acknowledgment

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References

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