determinations, by helium measurements, of magnetite from the upper portion of the Negaunee iron formation range from 800 million to 1650 million years and average 1300 million years (*7*). Since the pre-Cambrian flora with which we are dealing comes from near the base of the Gunflint formation, it seems likely that age may approach 2 billion years.

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Hemoglobin Regeneration Following Oral Administration of Chelated Iron

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Published evidence suggests that heavy metals attached to a chelate nucleus are available to plants but not to animal organisms. Stewart and Leonard (*1*) reported iron in the form of ferric potassium ethylenediamine tetraacetate to be readily utilized by chlorotic grapefruit trees when applied to the soil. On the other hand, Brendel *et al.* (*2*) administered several heavy metal-chelate combinations intraperitoneally to mice and obtained indirect evidence which led them to postulate that heavy metal ions attached to the chelate nucleus are not available to the animal body. We became interested in determining, by a direct experiment, whether or not this is true.

It is well known that extreme nutritional anemia can be induced in young rats maintained on a milk diet. The hematinic effect of iron added to the diet of the anemic rats is then clearly reflected in the rate of hemoglobin regeneration. Mitchell and Miller (*3*) showed that, in this procedure, the optimum daily iron requirement for a rat is about 0.25 mg if sufficient copper supplement is provided.

Fifty male, recently weaned, Sprague-Dawley rats were placed on a diet of powdered whole milk and water *ad libitum*. Blood hemoglobin determinations by the direct photometric method (*4*) were made on tail blood at intervals. At the end of 7 wk, the blood hemoglobin levels were uniformly low in more than two-thirds of the animals, and 30 of the most uniformly low animals were selected and divided into three groups of ten animals each.

Iron supplementation in the diet was then instituted in two of the groups. The first group received 0.1 mg iron per rat daily in the form of ferrous sulfate. The second group received the same daily dose of iron in the form of ferric sodium ethylenediamine tetraacetate (*5*). The third group received no iron and served as a control. These are suboptimal levels of iron intake, so intended in order to show possible differences in ab-

TABLE 1. Blood hemoglobin levels of anemic rats treated orally with ferrous sulfate and chelated iron.

Group	No. of animals	Form of iron	Average blood hemoglobin levels (g/100 ml) (5–26–53)	(6–9–53)	(6–23–53)	(7–8–53)	(7–22–53)	(8–3–53)
I	10	Ferrous sulfate	5.4 (± 0.4)*	8.2 (± 1.3)	10.5 (± 1.3)	11.0 (± 1.1)	11.4 (± 1.7)	12.3 (± 2.0)
II	10	Ferric sodium ethylenediamine	5.4 (± 0.5)	7.5 (± 1.3)	10.4 (± 0.5)	11.2 (± 0.8)	11.3 (± 1.1)	11.9 (± 1.8)
III	10	Control (no iron)	5.5 (± 0.4)	4.6 (± 0.5)	3.6 (± 0.4)	3.5 (± 0.3)	†	—

* Standard deviations.
† Controls in poor condition, two dead; see text for fate of remaining eight animals.

TABLE 2. Blood hemoglobin levels of anemic rats treated intravenously with saccharated iron oxide and chelated iron.

No. of animals	Form of iron	Average blood hemoglobin levels (g/100 ml) (7–15–53)	(7–22–53)	(7–29–53)	(8–3–53)	(8–12–53)
4	Saccharated iron oxide	3.5 (± 0.4)*	5.1 (± 0.4)	8.3 (± 0.2)	11.6 (± 1.4)	12.0 (± 1.5)
4	Ferric sodium ethylenediamine tetraacetate	3.5 (± 0.1)	4.2 (± 0.6)	4.6 (± 1.0)	5.4 (± 0.7)	5.2 (± 0.6)

* Standard deviations.

sorption or utilization of the two forms of iron. All three groups received 0.025 mg copper per rat daily as copper sulfate. Supplementation was carried on for a total of 9 wk. Blood hemoglobin determinations were made on tail blood at six different intervals during the supplementation period.

The average blood hemoglobin levels in Table 1 clearly indicate that the iron from the iron chelate was absorbed from the gastrointestinal tract and utilized for hemoglobin regeneration in the anemic rat at the same rate as iron from ferrous sulfate. Using the average figure of 6.7 ml of blood per 100 g body weight for the white rat, supplied by Cartland and Koch (*6*), and the generally accepted figure of 0.335 percent iron for the iron content of hemoglobin (*7*), an approximate calculation indicated that the rats were approaching 100-percent utilization of administered iron from both forms of iron during the early weeks of the experiment. The percentage utilized tapered off as the blood hemoglobins began to approach normal levels.

Fecal examinations were made several times during the course of the experiment. Feces from the ferrous sulfate group were usually slightly darker in color than those of the control group. Feces from the iron chelate group were usually softer and lighter in color than those of the controls. At autopsy, the lining of the stomach and gastrointestinal tract of the animals in both groups showed blackened areas. In the case of the iron chelate animals, this indicates that at least some of the complex was being broken down in the gastrointestinal tract, since the unbroken complex will not release iron to form sulfides of iron. Animals maintained on a milk diet with only the copper supplementation do not develop blackened areas in the gastrointestinal tract.

The effect of iron chelate administered parenterally was also explored in a preliminary way. When the control group had continued 6 wk beyond their depletion period on the iron-free diet, they had very low blood hemoglobin values, two had died, and the remaining eight were in poor condition. These eight were divided into two groups of four each, and intravenous therapy was instituted at a dosage level of 4 mg iron per kilogram twice weekly. One group received a solution of saccharated iron oxide (*8*). The other group received a sterile solution of 1.0 percent ferric sodium ethylenediamine tetraacetate (Table 2). Both groups continued to receive copper supplementation in the milk diet.

The poor hemoglobin response of the rats that received chelated iron intravenously is in accord with the postulate of Brendel *et al.* Once introduced into the body parenterally as the heavy metal complex, the metal ions remain attached to the complexing nucleus and are not readily available to the body. The parenteral results in turn lend further support to our suspicion that, following oral administration, iron is released from the chelate by some mechanism in the gastrointestinal tract before being absorbed. This mechanism is presently under further study.

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Studies of Copper Chlorophyllin-Odorant Systems[1]

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The use of chlorophyllins (water-soluble derivatives of chlorophyll) has been reported (*1*) for the effective reduction of breath odor, but no explanation of a mechanism by which such odor reduction is possible has been forthcoming in terms of the principles of physical chemistry.

By independent measurements, we have found that buffered (*2*) "solutions" of potassium copper chlorophyllin (*3*) will take up appreciable quantities of odorant, which can be estimated by titrating the solution with odorant to an olfactory end-point. In addition, we have measured the physical adsorption of gases onto powdered chlorophyllin (Table 1). In view of the marked difference between the two experimental techniques, the results are in surprisingly good agreement.

This observed adsorption is both physical and chemical, and where both occur, they may be of approximately equal magnitude. The physical adsorption is of the Langmuir type. Surface area by the BET method for several preparations of chlorophyllin is of the order of 15 m^2/g.

The fact that chlorophyllin does not form true water solutions but rather polyelectrolyte dispersions can be demonstrated by determinations of molecular-weight distribution and of intrinsic viscosity, and by precipitation with alcohol and with electrolytes. Chlorophyllin is about 75 percent nondialyzable through Cenco cellophane tubing; the particle weight is, therefore, primarily greater than 12,000. The particle weight of 3.6 percent of a sample is larger than 1-million as determined by sedimentation in a preparative centrifuge. The precipitation of chlorophyllin by ethanol increases linearly with the concentration of alcohol, and this suggests a continuous range of particle weights. Viscosity data (*4*) give straight-line functions for c/η_{sp} vs. $\sqrt{c}$ and fit a Fuoss Formulation giving $[\eta] = 0.33$; this indicates that particles are

[1] This paper was delivered at the meeting of Section C of the AAAS in Boston, Dec. 27, 1953.