tions have been made[4-6], but until now no experiment has been performed which might be analogous to the natural synthesis of fluoroacetate.

Our approach to this synthesis arose from consideration of 2 facts: only fluoroacetate, or compounds to which fluoroacetate might be readily converted in vivo, for example, fluoroacetone[7], fluorocitrate[8] or long chain ω-fluorofatty acids[9], have been characterized from plants. Secondly, chlorine may be introduced into organic molecules in cultures of *Caldariomyces fumago* by chlorodecarboxylation thus[10]:

$$\mathrm{HOOCCH_2CH_2COCH_2CO_2H} \xrightarrow[\text{chloro-peroxidase}]{\mathrm{Cl^-},\ \mathrm{H_2O_2}} \mathrm{HOOCCH_2CH_2COCH_2Cl}$$

In order to obtain fluoroacetic acid in a similar way, it is necessary that malonic acid - which is a universal constituent of plants - be fluoro-decarboxylated.

$$\mathrm{HO_2CCH_2CO_2H} \xrightarrow[[O]]{\mathrm{F^-}} \mathrm{HO_2CCH_2F} + \mathrm{CO_2}$$

Our first unsuccessful attempts to carry out this reaction involved using horseradish peroxidase and hydrogen peroxide. Subsequently we used sodium fluoride and malonic acid with either sodium hypochlorite or sodium peroxodisulphate as oxidising agents. In both cases after 4 days at 25 °C, the formation of fluoroacetate in small yield was readily detected by gas chromatography after conversion of the acid to the methyl ester. The methyl fluoroacetate formed had the same retention time and co-chromatographed with an authentic sample of methyl fluoroacetate on columns having apiezonal, methylsilicone gum, and polyethylene glycol adipate as stationary phases.

While it seems unlikely that either sodium hypochlorite or sodium peroxodisulphate is present in plants, our experiments demonstrate that fluoroacetate is readily synthesized, at ordinary temperature, from components, fluoride and malonic acid, which are present in plants which accumulate fluoride. Perhaps plants which biosynthesize fluoroacetate have evolved a fluoroperoxidase, analogous to the chloroperoxidase of *Caldariomyces fumago*.

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Insensitivity of the ferritin iron core to heat treatment[1]

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Summary. To test whether the reactivity of ferritin iron is affected by the heat treatment used in ferritin isolation, we prepared ferritin from the same horse spleen with or without heating. Both samples exhibited similar reactivity upon reduction or chelation of iron.

Colloidal aggregates of inorganic iron with sizes and properties similar to those of the ferritin core may be prepared by raising the pH or heating solutions of Fe(III)[2-6]. Over a period of 2 days at 25 °C, such synthetic iron polymers undergo a 'hardening' process in which they become denser, more colored, and more resistant to acid hydrolysis[7]. Current procedures for the isolation of ferritin call for heating tissue homogenate to 70–80 °C for 10 min to precipitate other proteins[8]. It is possible to prepare ferritin without heating[9], but there has been little reason to avoid the heat step. The observation of hardening of colloidal iron prompted the suggestion[7] that if ferritin were isolated without heating, it might be more reactive. The present work was undertaken to test this suggestion.

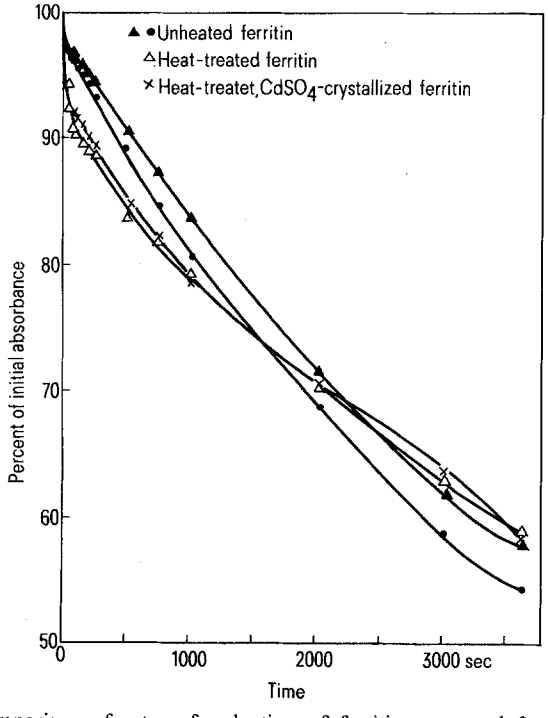
Methods. Horse spleen ferritin was isolated by 5 methods. The spleens were obtained on the day the animal died and were stored at 4 °C for 1 day before use. All operations were done at 4 °C. Method A. Spleen was ground in a meat grinder and homogenized for 30 sec in a Waring blender with 1.5 g H_2O/g spleen. The mixture was warmed to 70 °C over a period of ~6 min in a preheated vessel. It was maintained at 70 °C for 10 min and then cooled on ice. Following centrifugation and filtration to remove debris, ferritin was precipitated by addition of 300 g of ammonium sulfate per l while maintaining the pH at 7.0. The precipitate was isolated by centrifugation and dissolved in a minimal volume of water. Following centrifugation to remove insoluble material, and dialysis against 0.1 M Tris, pH 7.0, the supernatant was adsorbed onto a column of Bio-Rad Cellex-D anion exchange cellulose equilibrated with 0.1 M Tris, pH 7.0. The adsorbed material was eluted with 0.5 M KCl. After dialysis against 0.1 M Tris, pH 7.0, the red solution was centrifuged at 23,000 × g for 1 h and the supernatant recentrifuged at 100,000 × g for 3 h in a 5.5 cm long tube to pack $\gtrsim$ 95% of the ferritin iron. Pellets were dissolved in 0.1 M Tris, pH 7.0, and passed through an ascending column of Sepharose 6B (2.6 × 60 cm) eluted with the same buffer. The center of the single symmetrical protein peak was retained and dialyzed against 0.1 M KCl. Method B was the same as method A, except that the heating to 70 °C was omitted. Method C was the same as method A through the ammonium sulfate precipitation.

Mobilization of iron from ferritin by 0.05 M NTA at pH 7.4

Sample	Initial Fe/molecule	Reaction time (h)	Percent of Fe/mobilized
Spleen I:			
Heated ferritin (method A)	3030	47	8.0
Unheated ferritin (method B)	2860	47	10
Spleen II:			
Heated ferritin (method A)	2890	27	8.4
Unheated ferritin (method B)	2520	27	5.4

The redissolved precipitate was crystallized 3 times at 4 °C by addition of 5 g of $3CdSO_4 \cdot 8H_2O$ per 100 ml of solution. After each crystallization the product was dissolved in 0.1 M KCl. Attempts to use $CdSO_4$ to crystallize ferritin which had not been heated to 70 °C failed. Method D was the same as method A, except that 1 crystallization from $CdSO_4$ was used after the ammonium sulfate precipitation and the anion exchange step was omitted. The original homogenization was done with 0.02 M KCl instead of distilled water. Method E was the same as method D, but the heat step was omitted.

The purity of each ferritin preparation was checked by gel electrophoresis using 5% polyacrylamide gels. Only bands due to ferritin monomer, dimer and trimer were observed[10]. To further establish the purity of the samples, it was shown that the absorbance of ferritin at 420 nm (at which wavelength the iron core absorbs) could be eliminated by rabbit anti-horse ferritin antibody as described previously[12]. The absorbance remaining after immunoprecipitation was equal to that of the antibody solution alone. Protein was determined by the method of Lowry et al.[11] using crystalline bovine serum albumin as a reference. Ferritin iron was measured as described elsewhere[12]. To study iron mobilization by nitrilotriacetate (NTA), experiments were done in acid-rinsed 1 ml dialysis cells containing on one side 0.05 M NTA, 0.05 M KCl and 0.01 M N-2-hydroxyethylpiperazine-N'-ethanesulfonic acid, pH 7.4. The other compartment contained the same solution plus ferritin with an iron content of 100 µg. After vigorous shaking at 23 °C, 100 µl samples from the protein-free compartment were analyzed for iron by treatment with 0.5 ml of 0.14 M ascorbic acid in 0.13 M HCl. After 30 min, 0.5 ml of 1.0 M phosphate buffer (pH 2.2) containing 0.2% ferrozine (Sigma Chemical Co.) was added. The absorbance at 562 nm was measured after 45 min. These conditions eliminated interference by NTA. Blanks were prepared from dialysis cells run without ferritin.



Comparison of rates of reduction of ferritins prepared from a single horse spleen by methods B, A and C. The iron contents were 2520, 2890 and 2260 atoms per molecule, respectively. Reaction solutions contained 0.14 M thioglycolate, 0.0083 M EDTA and 0.083 M acetate (pH 4.8) at 23 °C. Absorbance was measured at 420 nm. EDTA alone mobilized about 4% of the iron in 1 h at pH 5.0. The 2 sets of data for unheated ferritin are representative of the reproducibility of the experiment.

Results and discussion. 2 spleens were fractionated by methods A, B and C, and 1 spleen was fractionated by methods D and E. The figure shows a typical comparison of the rates of iron loss from ferritin in the presence of the reducing agent, thioglycolate[13]. We observed no consistent differences in mobilization rates from heated, unheated, or $CdSO_4$-crystallized ferritin. It can be seen from the table that removal of iron by NTA[14] gave similar results. Unheated protein was somewhat more reactive than heated protein from 1 spleen, but the reactivities were reversed with a second spleen. Since we were unable to demonstrate any consistent difference between native ferritins isolated with or without heating, the use of heat-treated ferritin in studies of the iron core seems justified.

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