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4. The domes are hemispheres 1 m in diameter. They isolate a "map" area of approximately 0.785 m^2 and a water volume of approximately 0.262 m^3. The domes have a flange 5 cm wide about the rim. We achieved a satisfactory seal between the dome edge and the seafloor by gluing a strip of low-density foam rubber (10 cm thick) to the flange, loading it with three to eight 12-kg lead bricks (ordinarily four), and stuffing extra foam into any leaks. For one incubation on the reef crest, the dome was further secured with rock bolts. We evaluated the leakage by watching sediment movement within the domes during the incubations or, after the incubations, by injecting dye and then taking serial samples. In all but one experiment, leakage was judged to be negligible; results from that single experiment were discarded. Water samples were extracted with syringes through a serum cap port at the dome apex.
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Inducibility of Transferrin Receptors on Friend Erythroleukemic Cells

Abstract. *The ability of Friend erythroleukemic cells to bind transferrin and take up its iron increases substantially as a result of dimethyl sulfoxide–stimulated differentiation. Although transferrin-binding activity is also demonstrable in another mouse cell line of hematopoietic origin, the lymphoma cell, it does not increase on exposure to dimethyl sulfoxide. Gel filtration studies corroborate that the binding of transferrin to the erythroleukemic cells is due to the formation of a specific complex of transferrin and a membrane receptor. Thus, the specific interaction of transferrin with its receptor is another expression of dimethyl sulfoxide–induced differentiation in the Friend cell.*

Certain murine erythroleukemic cell lines, first isolated by Friend and coworkers (*1*), appear to behave like transformed erythroid precursor cells that are arrested at an intermediate stage of development. In culture, the cells resemble proerythroblasts and exhibit several macromolecules that are characteristic of erythroid cells. Among these are globin messenger RNA's (*2*), heme biosynthetic enzymes (*3*), carbonic anhydrase (*4*), and erythrocyte-specific membrane proteins (*5*). When grown in media supplemented with dimethyl sulfoxide (*6*) or various other chemical agents (*7*), the cells differentiate into forms that resemble orthochromatic erythroblasts and the amounts or activities of the characteristic erythrocytic macromolecules increase (*2, 5, 6*), hemoglobin becomes readily detectable (*6, 8*), and iron accumulates within the cells (*6*). Since transferrin is the major and perhaps the only source of iron for the biosynthesis of heme by erythroid cells (*9*), and the first stage in the interaction of transferrin with cells involves the binding of the protein to specific receptors on the cell surface (*9, 10*), we have studied the effects of growth in dimethyl sulfoxide on the capacity of erythroleukemic cells to bind transferrin. Although our measurements indicate that Friend cells have a significant number of receptors for transferrin prior to dimethyl sulfoxide treatment and hemoglobin accumulation, the capacity of the cells to bind transferrin and take up its iron is substantially increased during dimethyl sulfoxide–stimulated differentiation.

Studies with reticulocytes have shown that the binding of iron transferrin to its receptors is a time-, temperature-, and energy-dependent process (*10, 12*). A small amount of transferrin is adsorbed nonspecifically to reticulocytes at 4°C (*13*), but upon incubation at 37°C there is a progressive uptake of transferrin until a steady state is reached at 10 to 20 minutes (*10, 12*). This state is characterized by continued iron incorporation, but the amount of transferrin bound to the cell remains relatively constant. Thus, in the steady state the rate of binding of iron transferrin and the rate of release of iron-depleted transferrin are approximately equal. Mature erythrocytes appear to lack specific transferrin receptors as they exhibit only the nonspecific adsorption that occurs at 4°C (*14*).

In order to measure their transferrin-binding capacity erythroleukemic cells grown under various conditions were extensively washed with serum-free solutions and then incubated at 37°C in buffered saline containing glucose, bovine serum albumin (to minimize nonspecific adsorption of transferrin to cell membranes), and transferrin labeled with ^{125}I. Transferrin-binding capacity of the cells was studied with iron-saturated transferrin in order to maximize the binding to specific receptors (*11*). Attempts to study iron uptake with iron-saturated transferrin gave variable results, which we attribute to the presence of small amounts of iron nonspecifically bound to protein. In order to minimize this error, 60 percent saturated [^{59}Fe]-transferrin (*15*) was used to study iron uptake. In these experiments the size of the cells varied depending on the growth state of the culture and whether or not the growth medium was supplemented with dimethyl sulfoxide. Cultures approaching a stationary growth phase contained cells with a smaller average size than those growing exponentially. Likewise, cells grown in the presence of dimethyl sulfoxide for several days were smaller than cells grown in its absence. To correct for these effects, in each experiment a cell sample was taken for measuring the cell number and size (a Coulter model F counter calibrated with latex beads was used). Transferrin binding and iron uptake were expressed as micrograms bound per milliliter of cells.

The kinetics of transferrin binding to Friend erythroleukemic cells (clone 745) grown in the absence of or presence of dimethyl sulfoxide for 3 and 5 days is shown in Fig. 1. The time course of transferrin uptake by these cells is similar to that observed with reticulocytes, although the time required to reach a steady state is somewhat longer. Values obtained for transferrin binding to Friend

cells at steady state (60-minute incubation) are summarized in Table 1. Untreated cells (days 1 and 3) and cells treated with dimethyl sulfoxide for 1 day bound about 25 μg of transferrin per milliliter of cells. These cultures contained few (<1 percent) benzidine-positive hemoglobinized cells. Cultures grown for 3 and 5 days in the presence of dimethyl sulfoxide contained about 50 and 90 percent benzidine-positive cells, respectively. Cells from these cultures bound nearly three times as much transferrin on the basis of cell volume (about 70 μg per milliliter of cells), and also exhibited an increase in iron uptake. The increase in iron uptake from transferrin by dimethyl sulfoxide–treated cells is even more striking than the increase in transferrin-binding activity. After exposure to dimethyl sulfoxide for 1 day, the cells display a more than fourfold increase in their ability to remove iron from transferrin, while their transferrin-binding activity is still unaffected. By day 3, when transferrin-binding activity of induced cells has reached a maximum threefold increase over uninduced cells, iron uptake has increased nearly seven times. This dissociation between transferrin binding and iron uptake is perhaps not surprising, since the two functions are separate and distinguishable events in the transferrin-reticulocyte interaction (*10*). Furthermore, transferrin binding achieves a steady state when cellular receptors are saturated, while iron uptake increases with time as iron-depleted transferrin molecules are displaced by iron-bearing molecules. The increase in transferrin-binding capacity that occurs after growth in dimethyl sulfoxide is not due merely to reduction in cell size, since a similar increase was apparent when the results were expressed on a per cell basis. Cells grown in the absence of dimethyl sulfoxide for 5 days consistently exhibited a reduced transferrin-binding capacity, due perhaps to the fact that these cells had reached stationary growth phase about 2 days earlier and so may have been nutritionally depleted by day 5.

Table 1. Transferrin uptake of Friend erythroleukemic cells and other mouse cell types cultured in the presence and absence of dimethyl sulfoxide. The experimental details were the same as those in Fig. 1.

Cell type*	Culture period (days)	Dimethyl sulfoxide treatment	Mean cell volume (μ^3)	Transferrin bound† (μg/ml)	Iron uptake† (μg/ml)
Friend cell (745)	1	–	967 ± 23	28.9 ± 3.5 (4)	0.040 ± 0.003 (3)
		+	716 ± 18	27.2 ± 3.6 (5)	0.174 ± 0.060 (3)
Friend cell (745)	3	–	699 ± 33	24.6 ± 3.9 (9)	0.064 ± 0.010 (3)
		+	488 ± 39	73.9 ± 11.7 (5)	0.421 ± 0.014 (3)
Friend cell (745)	5	–	615 ± 11	13.2 ± 3.0 (4)	0.079 ± 0.009 (3)
		+	399 ± 19	68.3 ± 5.3 (5)	0.509 ± 0.033 (3)
Lymphoma (S49)	3	–	492	21.7	
		+	476	25.2	
Fibroblast (A9)	3	–	1478	5.03 ± 0.42 (3)	—
Erythrocyte		–	31	7.50 ± 4.06 (3)	—

*Fibroblasts were cultured in suspension with constant stirring in order to avoid trypsinization during harvesting. Erythrocytes were obtained from C57 mice. †Values (micrograms per milliliter of cells) represent the amount of transferrin binding and iron uptake ± the standard error after 60 minutes of incubation. The numbers in parentheses indicate the value of N used to calculate the standard error. Between two and six separate experiments were performed, in some cases in triplicate.

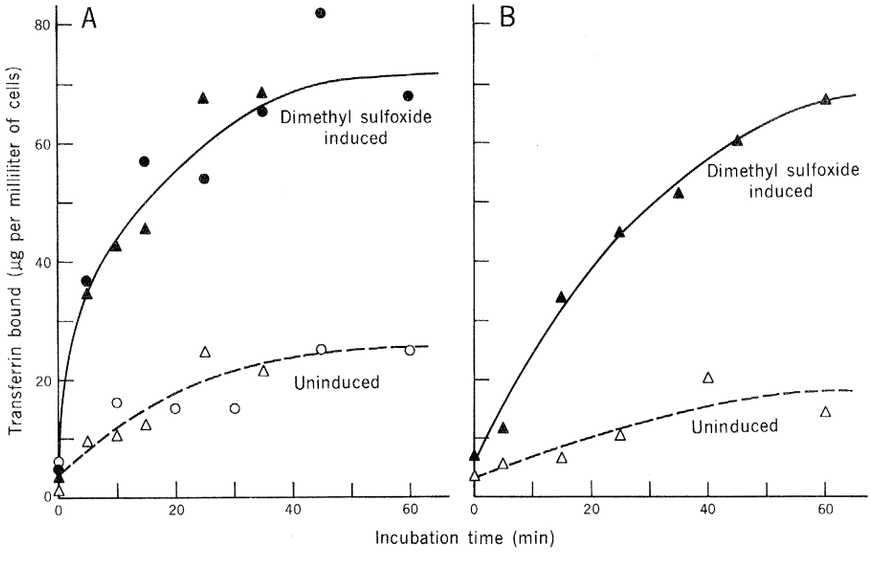

Fig. 1. Uptake of mouse transferrin by Friend erythroleukemic cells. Transferrin was prepared from frozen mouse serum (Pel-Freeze) by methods described for the purification of rabbit and mouse transferrin (*23*). The purified transferrin migrated as a single band on polyacrylamide-gel electrophoresis. The purified protein was labeled with ^{125}I (*24*). Friend erythroleukemic cells (clone 745) were grown in Dulbecco's modification of Eagle's medium, supplemented with 10 percent fetal calf serum, 100 units of penicillin per milliliter, and 100 μg of streptomycin per milliliter at 37°C in the presence or absence of 280 mM dimethyl sulfoxide. The cells were harvested by centrifugation at 600g for 5 minutes and washed at 4°C, once with growth medium without serum and three times with buffer A [0.13M NaCl, 0.005M KCl, 0.0074M $MgCl_2$, 0.01M Hepes [4-(2-Hydroxyethyl)-1-piperazineethanesulfonic acid], pH 7.5]. The cells were suspended in buffer A supplemented with 0.1 percent glucose and 0.2 percent bovine serum albumin (treated with Bio-Rad Chelex-100 to remove iron), and a portion was taken for determining the cell number and the mean cell volume (Coulter model F counter calibrated with latex beads). Reaction mixtures containing about 3×10^7 cells in 250 μl were first incubated at 37°C for 10 minutes. Transferrin was added to a final concentration of 0.5 mg/ml, and the incubation was continued at 37°C. At various intervals thereafter the reactions were terminated by addition of 4 ml of cold buffer A. The cells were washed at 4°C three times with buffer A and resuspended in buffer A, then portions were removed for determining cell number and radioactivity. (A) Cells cultured for 3 days in the presence (▲, ●) and absence (△, ○) of dimethyl sulfoxide (▲ and △ represent results of a single experiment; ● and ○ represent results from two other experiments). (B) Cells cultured for 5 days in the presence (▲) and absence (△) of dimethyl sulfoxide.

In order to investigate further the specificity of binding, we studied the transferrin-binding capacity of several other mouse cell types (Table 1). As expected, mouse erythrocytes bound much less transferrin than either treated or untreated erythroleukemic cells. Transferrin-binding capacity of a mouse fibroblast cell line (A9) (*16*) was also quite low when calculated on a volume basis, although on a per cell basis these larger cells appear to have about 40 percent of the number of receptors present on Friend cells. Uptake of transferrin by mouse lymphoma cells (S49) (*17*), however, was nearly equal to that of untreated Friend cells. In contrast to the Friend cells, which exhibited increased transferrin-binding capacity during dimethyl sulfoxide–stimulated differentiation, binding by lymphoma cells was unaffected by growth in the presence of dimethyl sulfoxide.

The nature of the complex containing bound ^{125}I-labeled transferrin was exam-

ined further by gel filtration chromatography (*18*). We have shown that, when a Triton X-100 extract of rabbit reticulocytes that had been incubated with [^{125}I]-transferrin is fractionated by gel filtration, two peaks having ^{125}I activity are obtained. The first peak, corresponding to a molecular weight near 450,000, is believed to represent a complex of transferrin with its receptor, while the second peak corresponds to free transferrin (*18*). Using the same techniques, we obtained qualitatively similar results with induced and uninduced Friend cells incubated with ^{125}I-labeled mouse transferrin. However, the free transferrin peak of uninduced cells was nearly ten times larger than that of induced cells, while the 450,000-molecular-weight peak of induced cells was about five times greater. These observations suggest that transferrin receptors are present in both uninduced and induced cells, but that the receptor-transferrin complexes of uninduced cells may be more labile than those of induced cells.

On the basis of the foregoing observations, we conclude that the Friend erythroleukemic cells contain specific cell surface receptors for transferrin prior to the acquisition of hemoglobin-synthesizing capacity. We estimate that untreated Friend cells have about 130,000 binding sites per cell, whereas after treatment with dimethyl sulfoxide each cell appears to have about 250,000 sites. By comparison, rabbit reticulocytes have been estimated to contain 200,000 to 560,000 binding sites per cell (*19*) and rat reticulocytes about 190,000 sites (*20*). Lymphoma cells displayed nearly as many transferrin-binding sites as the untreated erythroleukemic cells. Whether this is a general property of lymphoid cells remains to be established; the ability of lymphocytes to bind zinc transferrin suggests that this may be the case (*21*).

The transferrin-binding capacity of erythroleukemic cells increases about threefold on the basis of cell volume, or twofold on a per cell basis, as these cells differentiate, diminish in size, and accumulate hemoglobin in the presence of dimethyl sulfoxide. Whether this increase is a result of de novo synthesis of receptors is not known. Previous studies have indicated that transferrin receptors are metabolically stable in reticulocytes and that reticulocytes may be unable to synthesize transferrin receptors (*22*). The use of the Friend erythroleukemic cells cultured in dimethyl sulfoxide may provide an opportunity to study the metabolism of these surface receptors during erythroid differentiation.

Note added in proof: Three variant erythroleukemic cell lines, unable to produce hemoglobin in response to dimethyl sulfoxide, also failed to increase transferrin-binding capacity in the presence of this agent.

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Cubanite: A New Sulfide Phase in CI Meteorites

Abstract. *Cubanite ($CuFe_2S_3$), previously unobserved in meteorites, has been discovered in two carbonaceous chondrites, Orgueil and Alais. The association of this mineral with low-copper pyrrhotite suggests that it formed in a low-temperature environment on the meteorite parent body.*

Carbonaceous meteorites in class CI (*1*) have a unique mineralogical makeup. Phases which occur in these objects include fine-grained phyllosilicate minerals (which constitute a major portion of the meteorites), carbonates, oxides, anhydrous silicates, and sulfur in three oxidation states: as elemental sulfur, sulfides, and sulfates. This assemblage of minerals, and the approximately solar element abundances reflected in the CI bulk composition, have led to considerable speculation about the environment of their formation. There are basically two schools of thought on the matter. One view (*2*) is that most of the observed minerals were produced by reaction of previously condensed solids with a cooling (below approximately 700°K) gas in the nebula. According to the second view (*3, 4*), alteration of preexisting phases on a parent body, possibly in the presence of liquid water, is responsible for the CI mineral suite. We report here the presence of a hitherto unobserved sulfide, cubanite ($CuFe_2S_3$), in two CI meteorites, Alais and Orgueil. The occurrence of this mineral, and its relationship to other phases present, may add some constraints to the discussion of conditions during the formation of these meteorites.

We first observed cubanite in the sink fraction of a methylene iodide density separate of Orgueil. In both Orgueil and Alais it exhibits a distinctive stubby prismatic habit (Fig. 1) and is easily differentiated visually from the predominant sulfide of these meteorites, pyrrhotite (*5*), which occurs as hexagonal plates. Crystal faces of the separated cubanite grains show little tarnish or corrosion, in contrast to the pyrrhotite which frequently has a frosted appearance under the binocular microscope and often exhibits pitted crystal faces at higher magnifications. Results of electron microprobe analyses of cubanite separated from both Alais and Orgueil are shown in Table 1. The mineral is stoichiometric