to be accumulated together in tuna.

Since tuna has a substantial content of Se, the question arises of whether this amount of Se is in itself a hazard, or whether its presence might reduce the toxicity of Hg. The content of tuna added to the diet in these experiments supplied approximately 0.3 to 0.6 ppm of Se. This is a desirable nutritional content and is roughly an order of magnitude less than the content at which signs of toxicity appear (*5*). Since the growth of quail on the tuna diets was comparable to that on corn-soya diets (Table 1), no evidence exists that this content of Se was harmful.

Direct evidence that dietary Se can decrease the toxicity of methylmercury was obtained in a study in which rats received a purified basal diet containing 20 percent casein or the same diet supplemented with 0.5 ppm of Se, with various concentrations of methylmercury added to the drinking water (Fig. 1). The basal diet was quite low in Se content (approximately 0.02 ppm) and also lacked vitamin E, since the experiment was designed to test whether Hg might induce signs of Se–vitamin E deficiency in a manner similar to that observed with Ag (*6*). Although Se produced a slight growth response when added to the basal diet, no signs of Se or vitamin E deficiency were observed in any of the groups tested. Mercury depressed growth at all concentrations, and Se improved growth at all concentrations of Hg, the effect being most evident at the higher concentrations of Hg. The most interesting finding, however, was that all the rats fed 10 ppm of Hg without Se had died, but those fed the same diet with Se were still alive at the end of week 6. In the groups receiving 25 ppm of Hg, all animals were dead by week 6, but the addition of Se increased the survival at week 4.

The suggestion that Se in tuna may reduce the toxicity of Hg is supported by studies from Parizek's laboratory showing that Se reduces the acute toxicity of $HgCl_2$ injected into rats, and may complex with Hg in the blood to decrease the availability of each element (*7*). The biological availability of Se in tuna is only 50 percent of that found for other sources of Se, even though comparable concentrations of Se in the blood are attained regardless of the source (*8*); it may be no coincidence that molar ratio of Hg to Se in tuna approaches 0.5.

Selenium, like S, readily complexes with Hg, and both Se and Hg tend to be associated with S in proteins. It is thus reasonable to expect that Se and Hg might occur together, as a result of their affinity for each other or their common affinity for S. The content of Se in the oceans is about 0.09 part per billion (*9*), approximately the same as that of Hg (*9, 10*); thus both elements are strongly accumulated by tuna. In addition to Se, other substances in tuna might also affect Hg accumulation or toxicity. The observation (*10*) that individuals can have elevated concentrations of Hg in the blood and yet be free of symptoms of Hg poisoning suggests, as one possibility, that agents modifying toxicity may indeed be operative.

The implications of this study are that the danger for man of Hg in tuna may be somewhat less than anticipated, and that the total Hg contents in the diet or even in the blood may not be valid criteria because of the presence of modifying factors. Clearly, further research on these matters is needed.

H. E. GANTHER
Department of Nutritional Sciences, University of Wisconsin, Madison 53706
C. GOUDIE
M. L. SUNDE
Department of Poultry Science, University of Wisconsin
M. J. KOPECKY
Department of Nutritional Sciences, University of Wisconsin
P. WAGNER, SANG-HWAN OH
W. G. HOEKSTRA
Departments of Nutritional Sciences and Biochemistry, University of Wisconsin

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Chalcocite Oxidation and Coupled Carbon Dioxide Fixation by Thiobacillus ferrooxidans

Abstract. *The reaction of cell suspensions of* Thiobacillus ferrooxidans *with pulverized chalcocite (Cu_2S) in a Warburg manometric apparatus resulted in oxygen uptake accompanied by increased solubilization of copper and fixation of carbon dioxide. Since the only detectable oxidized products were cupric ions and the more oxidized form of the sulfide mineral, that is, digenite or covellite, the apparent source of energy for the carbon dioxide fixation was provided by the oxidation of the cuprous copper of the chalcocite.*

Thiobacillus ferrooxidans, a chemolithoautotrophic bacterium, obtains energy for CO_2 fixation and growth by oxidizing certain reduced forms of sulfur or Fe^{2+} (*1*). Furthermore, the oxidation of certain sulfide-containing minerals yields energy for CO_2 fixation (*1*) and usually produces SO_4^{2-} accompanied by a lowering of the *p*H of the reaction mixture. Fox (*2*) noted an increased *p*H when growing *Ferrobacillus (Thiobacillus) ferrooxidans* on chalcocite (Cu_2S) and speculated therefore that SO_4^{2-} was not a product of chalcocite oxidation and that the small amount of growth observed resulted from the energy made available by the oxidation of Cu^+ to Cu^{2+}. We pre-

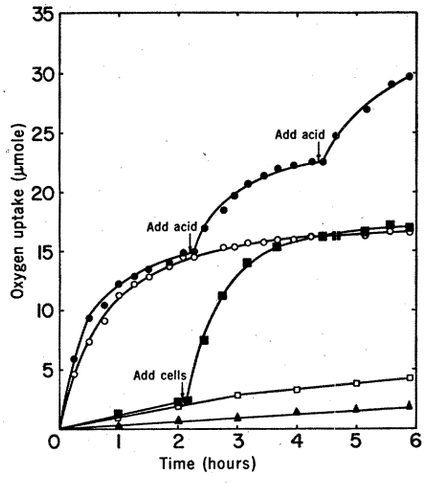

Fig. 1. Chalcocite (Cu_2S) oxidation by cell suspensions of *T. ferrooxidans*. Each Warburg flask contained 75 mg of chalcocite (325-mesh), 0.5-ml cell suspension (as indicated in Fig. 1), 0.1*N* H_2SO_4 added to adjust the *p*H to 1.7 initially and additional 50-μmole portions (as indicated in Fig. 1), and 5 μmole of KH_2PO_4. Total volume, 2.0 ml; temperature, 31°C; ●, all constituents present at 0 time, 50 μmole of H_2SO_4 added at 120 minutes and at 270 minutes; ○, all constituents present at 0 time, no additions; ■, no cells at 0 time, cells added at 120 minutes; □, no cells; and ▲, boiled cell suspension added at 0 time.

sent here results which show that heavy cell suspensions of *T. ferrooxidans* do indeed catalyze the oxidation of Cu^+ in chalcocite to Cu^{2+}, and that the oxidation is accompanied by CO_2 fixation.

The culture of *T. ferrooxidans*, obtained from a copper mine operated in Arizona by Cities Service Corporation, was cultured and maintained as previously described (*1, 3*). Cell suspensions were prepared by resuspending centrifuge-sedimented cells in 0.01*N* H_2SO_4 to a cell density equivalent to about 1.0 mg of cellular nitrogen per milliliter and were used within a few weeks of the time of preparation.

A museum-grade specimen of chalcocite, shown by mineralogical analysis (*4*) to be predominantly chalcocite and whose chemical composition was 76.5 percent copper, 19.1 percent sulfur, and 0.17 percent iron, was ground to pass through a 325-mesh sieve. We followed its oxidation using manometric methods with Warburg-type reaction vessels. The mineral and KH_2PO_4 were placed in the flask, and 0.1*N* H_2SO_4 was added to adjust the *p*H to a final value of 1.7. Cell suspension and $[^{14}C]NaHCO_3$ were added from sidearms after an initial period of equilibration. Control flasks contained either no cells, no mineral, or boiled cell suspensions. The presence of KOH in the center well had no effect on the observed amount of O_2 absorbed. Oxygen uptake was measured, and, at the conclusion of the experiment, the reaction mixtures were placed in small tubes and flushed with a mixture of $[^{12}C]CO_2$ and air at 0°C until the ^{14}C count, as determined by liquid scintillation spectrometry (*5*) on samples taken at various times, became constant.

The reaction mixtures were then acidified with 5 ml of 0.01*N* HCl, diluted to a known volume (about 10 ml), and filtered through a 0.45-μm Millipore filter. Concentrations of Cu^+ and Cu^{2+} in the filtrate were determined by the 2,2′-biquinoline method (*6*). The concentration of SO_4^{2-} was estimated by an isotope dilution method. Sulfuric acid ($[^{35}S]H_2SO_4$) was added to the Warburg flask at the start of the experiment, and the specific activity (counts per minute of ^{35}S per milligram of $BaSO_4$) of SO_4^{2-} was determined after the dried $BaSO_4$ was weighed and the ^{35}S radioactivity was counted with a Geiger-Müller tube. The addition of more than 2 μmole of SO_4^{2-} caused a significant decrease in the specific activity. Thus, the formation of amounts of SO_4^{2-} larger than this could be easily detected and measured.

Figure 1 shows the O_2 uptake observed in a typical experiment with chalcocite. The rate of oxidation was initially rapid, decreased with time, and finally approached zero; the rate of oxidation was restored to near its initial value by the addition of H_2SO_4. The *p*H of the reaction mixture during the oxidation period increased from 1.7 to about 4.6. Cells showed no oxidative activity at initial *p*H values of 4.6 or above. Although the data in Fig. 1 show that controls containing boiled cells or no cells exhibited little O_2 uptake, there was some solubilization of copper in the controls shown in Table 1. This solubilization was due to the acid solubility of the chalcocite mineral and occurred immediately upon treatment with acid.

The acid-solubilized Cu^+ oxidized spontaneously under the conditions of these experiments. The rate of the nonbiological reaction was so high that we were unable to demonstrate biological oxidation as measured by CO_2 fixation using a solution of CuCl as the substrate. Accompanying the spontaneous oxidation of Cu^+, there is a stoichiometric uptake of O_2. This finding accounts for the small amount of O_2 absorbed by the control with no cells shown in Fig. 1. The control flask with boiled cells, as shown in Table 1, did contain some reduced form of copper. We have observed this result consistently in our experiments. Failure of the small amount of acid-solubilized Cu^+ to be completely oxidized in the presence of inactive cells may be due to its chelation by some cell constituents.

Table 1 summarizes results from several experiments. The data show that active cells act on chalcocite with the uptake of O_2. Although CO_2 was fixed and copper was solubilized, no SO_4^{2-} was detected after chalcocite oxidation. The ratio of O_2 consumed to microbially solubilized copper is 0.43. In other experiments we noted that this value ranged from about 0.42 to 0.51; it is in reasonably good agreement with that predicted according to the equation

$$2Cu_2S + 2H_2SO_4 + O_2 \rightarrow 2CuS + 2CuSO_4 + 2H_2O$$

Mineralogical analysis of the mineral residue after extensive microbial oxidation of chalcocite has shown the presence of large quantities of digenite (Cu_9S_5) and covellite (CuS). This finding is in agreement with observations

Table 1. Chalcocite (Cu_2S) oxidation by cell suspensions of *Thiobacillus ferrooxidans*. Experimental conditions are the same as those for Fig. 1 with additions of either 30 μmole of $[^{14}C]NaHCO_3$ (18,000 counts per minute per micromole) or 50 μmole of $[^{35}S]H_2SO_4$ (1500 counts per minute per micromole); time, 3 hours; ^{35}S and ^{14}C determinations could not be made on the same reaction mixture; —, not determined; none, less than the experimental error for the specific determination, that is, 0.01 μmole for $[^{14}C]CO_2$, 2.0 μmole for SO_4^{2-}, and 0.1 μmole for Cu^+.

Experimental conditions	O_2 consumed (μmole)	CO_2 fixed (μmole)	Soluble products formed		
			Copper		SO_4^{2-} (μmole)
			Cu^+ (μmole)	Total (μmole)	
Active cells	19.3	0.29	None	54.0	—
Boiled cells	0.2	None	4.8	14.5	—
Active cells	23.6	—	None	52.8	None
Boiled cells	1.5	—	—	15.0	None
Active cells, no Cu_2S	None	None	—	—	None

from another laboratory which recently came to our attention (*7*).

The substantial amount of CO_2 fixation is of great interest. The efficiency of fixation, as expressed by the ratio of the number of micromoles of CO_2 fixed to each 100 μmole of O_2 uptake, was about 1.5, which is very nearly the same as that for the oxidation of Fe^{2+} (*1*). The quantity of O_2 absorbed represents that necessary for accepting the electrons released during the oxidation of the copper of chalcocite.

Our interpretation of these results is that *T. ferrooxidans* does oxidize the Cu^+ of chalcocite to Cu^{2+} and is capable of using the energy resulting from this oxidation for CO_2 fixation. Other possible sources of energy for the observed fixation are the oxidation of the sulfur moiety of the chalcocite to some form less oxidized than SO_4^{2-} or the cyclic oxidation of the small amount of iron found in the mineral. We have been unable to find in the final reaction solutions any reduced form of sulfur such as S^0, SO_2, or polythionates. If S^0 were formed, the cells would immediately oxidize it to SO_4^{2-} and the efficiency of CO_2 fixation would rise to about 5.0. Therefore, we believe that the oxidation of the sulfur moiety of chalcocite to such incompletely oxidized forms of sulfur was not the energy source for the observed CO_2 fixation.

There is iron in the chalcocite sample that was used—about 2 μmole per flask—but not over 5 percent of the chalcocite was oxidized in these experiments. Therefore, only 0.1 μmole of soluble iron would be present at any time. The low rate of oxidation expected for this small amount of iron would seem to preclude its being a significant factor as a source of energy for the large amount of CO_2 fixation that occurred. However, presently available evidence does not exclude the possibility that the cyclic oxidation of even the small amount of iron present may be the source of some or all of the energy necessary for the observed CO_2 fixation.

ALLEN M. NIELSEN
JAY V. BECK

Department of Microbiology,
Brigham Young University,
Provo, Utah 84601

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Thymus-Derived Lymphocytes: Humoral and Cellular Reactions Distinguished by Hydrocortisone

Abstract. *Lymphocytes derived from the thymus (T cells) take part in the induction of humoral antibody and also effect cell-mediated graft-versus-host reactions. Preliminary treatment of mice with hydrocortisone caused an inhibition of T-cell function in humoral immunity, while enhancing the graft-versus-host reactivity of the same population of spleen cells. This suggests that different types of T cells participate in cellular and humoral immune reactions.*

Lymphocytes derived from the thymus (T cells) play a central role in both cellular and humoral immune responses. The T cells apparently act directly in such cellular reactions as delayed hypersensitivity (*1*), contact-dependent cytotoxicity to target cells (*2*), and graft-versus-host reactions (*3*). In contrast, humoral immunity is mediated by circulating antibodies secreted by lymphoid cells that are not derived from the thymus (B cells). Nevertheless, the induction of specific antibody by B cells against many antigenic determinants requires the cooperation of T cells (*4*). The mechanism by which T cells and B cells interact during induction of the antibody response is not clear. However, it is known that B cells may not produce antibody to haptenic determinants on an immunogen unless other determinants on the immunogenic molecule are recognized by T cells. The part of the immunogen that interacts with T cells is called the carrier. Thus, induction of antibody synthesis in B cells, against certain haptenic determinants, requires the interaction of T cells with the carrier portion of the immunogen (*5*). In addition, the interaction between the T cell and the carrier, needed for the induction of antibody to hapten, does not appear to require the production of antibody to the carrier antigens themselves (*6*). Hence T cells serve as independent helpers in humoral immunity, as well as agents of cellular immunity. We have designed experiments to determine whether the T cells which mediate cellular immunity are the same T cells which act as helpers in humoral immunity.

The cells which induce graft-versus-host reactions, in mice, are resistant to prior treatment of the animal with hydrocortisone (*7*, *8*). Our experimental approach, therefore, was to give mice prior treatment with hydrocortisone and to measure the ability of their cells to perform both graft-versus-host reactions and humoral immunity functions, which are dependent on T cells. We found that T cells which perform a helper function in antibody responses are separable from T cells that are involved in a cell-mediated graft-versus-host reaction.

We measured T-cell helper function by inducing a primary antibody response in tissue culture against a hapten conjugated to a carrier (*5*). Formation of antibodies to hapten by B cells in this system in vitro depends upon successful prior sensitization of T cells in vivo against the specific carrier. Therefore, induction of antibodies to hapten serves as a sensitive measure of specific T-cell helper function. We used the dinitrophenyl (DNP) group as hapten, and rabbit serum albumin (RSA) as carrier (*9*). Ten male mice of strain C57BL/6j received a 0.1-ml intraperitoneal injection of 2.5 mg of hydrocortisone acetate. Ten control mice were injected with buffer alone. Two days later the mice were injected intraperitoneally with 200 μg of RSA in complete Freund's adjuvant (Difco). The spleens of the animals were removed after an additional period of 2 days. Part of each spleen was assayed for graft-versus-host reactivity (*10*) and part was used in the Millipore filter well technique for the induction of an antibody response to DNP in vitro (*5*, *11*, *12*). Filter wells contained slices of spleens with 0.01 ml of medium that contained 50 μg of DNP conjugated to RSA. Forty-eight hours later the culture medium was replaced with antigen-free medium. This medium was