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.

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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

collected 4 days later and assayed for the presence of antibodies to DNP by the modified T4 bacteriophage method (*12, 13*). The DNP was conjugated to T4 bacteriophage and the presence of antibodies to DNP was detected by incubating the test medium with the combination of DNP and bacteriophage. The inactivation of bacteriophage and the subsequent reduction in the number of plaques produced by bacteriophage on cultures of *Escherichia coli* served as a sensitive assay of antibodies to DNP. A control medium was prepared by incubating spleen slices without added antigen. The activity of B cells was studied by incubating spleen slices with DNP conjugated to poly-L-lysine (DNP-PLL). This immunogen appears to be independent of the thymus and in some studies was found to induce B cells to produce antibodies to DNP without the help of T cells (*14*).

The results of one experiment are illustrated in Fig. 1. Both hydrocortisone-treated mice and untreated mice produce antibodies to DNP in vitro when exposed to DNP-PLL. This is shown by the steep slope of inactivation of the combination of DNP and bacteriophage in Fig. 1. Thus, preliminary treatment with hydrocortisone by itself does not inhibit the ability of B cells to produce antibodies to DNP. Only spleens derived from mice not treated with hydrocortisone were able to respond to the immunogen DNP-RSA that is dependent on T cells. This indicates that preliminary treatment with hydrocortisone inhibited the helper function of the T cells that is necessary for interaction with the RSA carrier. Similar results were obtained when mice were injected simultaneously with RSA and hydrocortisone, and then tested for RSA carrier effect in vitro.

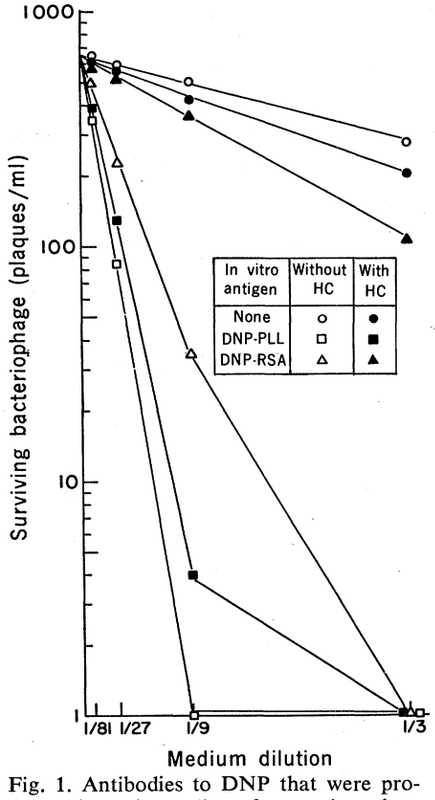

Fig. 1. Antibodies to DNP that were produced by spleen slices from mice given carrier antigen after prior treatment with hydrocortisone (HC). Mice were injected with carrier antigen (RSA) with or without prior treatment with hydrocortisone. Two days later spleen slices from the ten mice were incubated in vitro with medium alone, with thymus-independent DNP-PLL, or with thymus-dependent DNP-RSA antigens. The slope of each curve reflects the amount of antibody in the medium pooled from each group (*12, 13*).

To test the hydrocortisone sensitivity of T cells after first immunizing them against carrier antigens we injected mice with RSA and then treated them with 2.5 mg of hydrocortisone 5 days later. Control mice did not receive hydrocortisone. The interaction between DNP and the RSA carrier, dependent on T cells, remained intact in these experiments. (Results of one of these studies are illustrated in Fig. 2.) Therefore, the T cells which act as helpers develop resistance to hydrocortisone after undergoing primary sensitization to RSA.

The ability of the C57BL/6j spleen cells to initiate graft-versus-host reactions was assessed by slightly modifying the method for assaying this reaction in the rat (*10*). Parental or syngeneic spleen cells were suspended in saline buffered with phosphate, *p*H 7.2, and 0.05 ml of the suspension, containing 4×10^6 cells, was injected into the right foot pads of 1-month-old (C57-BL/6j $\times$ C3H)F_1 mice. Each group was composed of five mice. The mice were killed after 1 week and the weight of the right popliteal lymph node that was draining in each mouse was compared to the weight of the left lymph node that was the control. A positive graft-versus-host reaction was indicated by a statistically significant increase in the relative weights of the right lymph nodes as compared to those of the left lymph nodes. Control animals, injected with comparable numbers of syngeneic cells, did not demonstrate an increase in the weight of the draining node. Preliminary treatment with hydrocortisone increased the graft-versus-host reactivity of the same parental spleens in which the helper function was inhibited. The average ratio of weights from right and left nodes was 3.2 : 1 in the group of mice which received parental spleen cells that were treated with hydrocortisone. The average ratio was 1.9 : 1 ($P < .05$) in the group of mice that received parental spleen cells that were not treated. An assay with spleen weights to measure the graft-versus-host reaction also indicated that preliminary treatment with hydrocortisone increased the relative graft-versus-host activity of spleen cells (*8*).

Our results support the following conclusions:

1) The graft-versus-host reaction, as observed by others (*7, 8*), depends upon a population of T cells which are resistant to hydrocortisone.

2) The B cells which synthesize antibodies to hapten are resistant to hydrocortisone. This was demonstrated by the production of antibodies to DNP in response to DNP-PLL which was unaffected by hydrocortisone treatment.

3) The T cells that interact with carrier antigens to serve the helper function are susceptible to hydrocortisone.

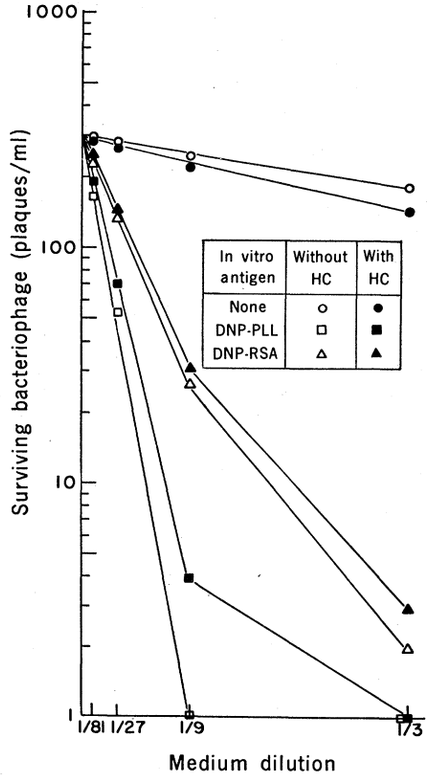

Fig. 2. Antibodies to DNP that were produced by spleen slices from mice given carrier antigen before treatment with hydrocortisone (HC). All the mice were injected with carrier antigen (RSA) and 5 days later the experimental group was injected with hydrocortisone. Spleen slices were prepared 2 days later.

This was shown by failure of spleen cells, first treated with hydrocortisone, to recognize RSA as a carrier for the DNP hapten.

4) The T cells involved in helper function acquire resistance to hydrocortisone after they have been sensitized to carrier antigens. We found that injecting RSA before treatment with hydrocortisone preserved the carrier effect. This may explain why the suppression of antibody formation by adrenal steroids is much less effective when these drugs are administered after antigen stimulation (*15*).

The different susceptibilities to hydrocortisone provide evidence that both the graft-versus-host and helper functions that are dependent on the thymus are controlled by different T-cell mechanisms. It is possible that the thymus can program the differentiation of precursor lymphoid cells into two completely separate populations—a population resistant to hydrocortisone that mediates graft-versus-host and possibly other cell-mediated reactions, and a population sensitive to hydrocortisone that provides the helper function in humoral immunity. On the other hand, as suggested by Raff and Cantor (*16*), different immunologic activities may be performed by T cells as they pass through stages of differentiation. Thus, T cells at an early stage of differentiation might be active as helper cells and mediate graft-versus-host reactions only after further maturation. A third possibility is that the same T-cell type has both functions, but that the mechanisms involved in carrier interaction alone are susceptible to hydrocortisone. This possibility appears less likely because the carrier effect itself is resistant to hydrocortisone once the T cell is sensitized (Fig. 2). Moreover, hydrocortisone appears to increase the graft-versus-host potential of lymphocyte populations by destroying susceptible lymphocytes rather than by merely altering cell functions (*8*). It is unlikely that specific tolerance to RSA could have been induced by injecting RSA in complete Freund's adjuvant.

We have studied the effects of hydrocortisone on the development of lymphocytes which mediate contact-dependent lysis of target cells (*17*). Preliminary treatment of lymphocytes in vitro with hydrocortisone reduced the number of lytic lymphocytes developing after sensitization. The simultaneous presence of sensitizing antigens appeared to prevent this inhibitory effect. Hence, T cells that are needed to produce cytolysis of target cells may also differ from T cells active in the graft-versus-host reaction. In addition to different susceptibilities to hydrocortisone, T cells active in graft-versus-host reactions and T cells active in cytolysis differ in their response to antibodies against a T-cell antigen (*18*). Our conclusions pertain to peripheral T cells. Studies of the antibody response of mice to sheep red blood cells suggest that lymphocytes in the thymus may produce a carrier effect that is resistant to corticosteroids (*19*).

Therefore, at least two, and possibly three, different T cells may be distinguished. Identification of these cell types is important to our understanding of the basic processes of lymphoid cell differentiation and cooperation which are factors both in antibody production and in cell-mediated immune responses. Competition between humoral antibodies and immune lymphocytes may lead to enhanced tumor growth in man (*20*) or to allograft tolerance (*21*). Therefore, identification and separation of lymphoid cell types ultimately may provide a way of manipulating and controlling immune reactions in patients with tumors or transplanted organs.

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Pancreatic Beta-Cell Web: Its Possible Role in Insulin Secretion

Abstract. *A cortical band of fine microfilaments is consistently observed in the beta cells of the rat pancreas. Alteration of this cell web by cytochalasin B is associated with an enhancement of glucose-induced secretion of insulin by isolated islets. The microfilamentous web of the beta cell may play an important role in the emiocytosis of insulin secretory granules, by controlling their access to the cell membrane.*

In 1968, Lacy *et al.* (*1*) suggested that the integrity of the microtubular-microfilamentous system of the pancreatic beta cell is required in order that glucose exert its stimulant action on insulin release. Further investigations (*2*) again showed that both mitotic-spindle inhibitors (colchicine and vincristine) and microtubule stabilizers (D_2O and hexylene glycol) inhibit or suppress in vitro glucose-induced insulin secretion. We now report on a previously neglected component of the microtubular-microfilamentous system and its possible role in the insulin secretory process.

Our study was undertaken because, in the course of recent ultrastructural studies on the pancreatic beta cell, we became aware of the existence of a