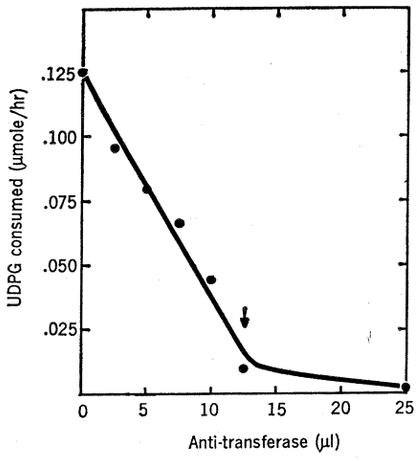

Fig. 2. Normal transferase activity remaining in the supernatant after precipitation of the enzyme with increasing amounts of antitransferase. Replicate portions of 300 μg of normal red cell protein were mixed with increasing amounts of antitransferase in a final volume of 150 μl. The tubes were incubated for 1 hour at 37°C and 2 days at 4°C, centrifuged, and 50 μl of supernatant assayed for transferase activity by uridine diphosphoglucose (*UDPG*)–consumption assay. The arrow indicates the amount of antitransferase used for the data in Fig. 3.

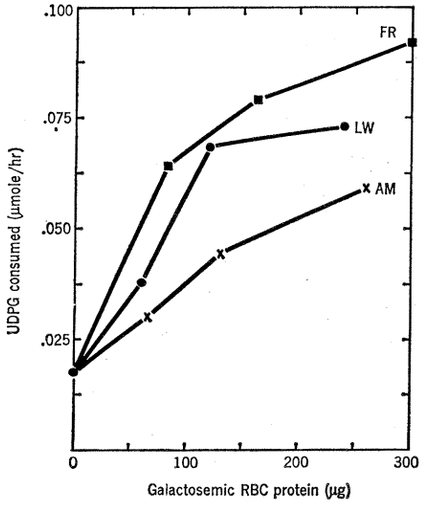

Fig. 3. Normal transferase activity remaining in the supernatant after precipitation with a constant amount of antitransferase that was first treated with increasing amounts of galactosemic red cell protein from three different galactosemics. Portions of galactosemic red cell protein as indicated were mixed with a constant amount (12.5 μl) of antitransferase in a volume of 100 μl. The tubes were incubated at 37°C for 1 hour and 4°C for 3 days, centrifuged, and 300 μg of normal red cell protein in 50 μl was added to each tube. After incubation as before, the tubes were centrifuged and 50 μl of supernatant was assayed for transferase activity by uridine diphosphoglucose (*UDPG*)–consumption assay. *AM*, 3-month-old male Caucasian galactosemic; *FR*, 3-year-old male Caucasian galactosemic; *LW*, 3-year-old female Negro galactosemic; *RBC*, red blood cell.

transferase activity. The removal of transferase activity from the supernatants is proportional to antibody concentration (Fig. 2).

To confirm the presence of a cross-reacting material (CRM) to transferase enzyme protein in the galactosemic preparations, increasing amounts of galactosemic red cell protein were mixed with preparations having a constant amount of the antibody (12.5 μl) determined by the precipitation data in Fig. 2. After 1 hour at 37°C and 3 days at 4°C the tubes were centrifuged and a constant amount of normal (Gt^+/Gt^+) transferase activity identical with that used for the data of Fig. 2 was added to each tube. The tubes were incubated as before and the transferase activity remaining in the supernatant was then determined. The CRM transferase protein in the galactosemic erythrocyte preparations formed a precipitin with the same antibody that complexes with the normal, active enzyme (Fig. 3).

Whole immune serum before fractionation on Sephadex G-200 as well as serum obtained from the same rabbit before immunization did not precipitate transferase activity. However, the whole immune serum, before fractionation, does form precipitin bands in double immunodiffusion gels against transferase protein whereas the control serum does not.

The identification of a CRM transferase protein in galactosemic cells indicates that the nature of the defect in this enzyme-deficiency disease is a "point" mutation which renders the enzyme protein catalytically inactive but has little or no effect on the antigenic properties of the molecule. The possibility was previously suggested by studies with antitransferase prepared with calf liver enzyme (*5*), and is further supported by the report on interallelic complementation at the transferase locus in which cultured fibroblasts from certain patients with galactosemia produced active transferase after hybridization (*6*).

It is reasonable to approach the therapy of inborn errors of metabolism in which the involved enzyme protein is present, albeit malfunctioning, by attempts at activation or stabilization of the affected molecule. It is therefore important to identify such diseases where possible; such an identification has now been made of galactosemia.

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Does DDT Inhibit Carbonic Anhydrase?

Abstract. *At a concentration of 50 to 100 micrograms per milliliter,* p,p'-*DDT (and* p,p'-*DDE) did not inhibit the rate of hydration or dehydration of carbon dioxide by carbonic anhydrase. At concentrations greater than 500 micrograms per milliliter, partial inhibition of the rate of dehydration of carbonic acid was observed, but this involved precipitation of drug in the reaction vessel. This degree of inhibition suggests that DDT may not inhibit carbonic anhydrase effectively at the usual concentrations found in tissue after exposure of organisms to DDT in the environment.*

Birds of prey have been found to lay thin-shelled eggs, a phenomenon which has been ascribed by Peakall (*1*) and by Bitman *et al.* (*2*) to inhibition of carbonic anhydrase in the shell gland by DDT (*3*). No direct studies of inhibition were done; the shell gland or oviduct of treated birds had about 60 percent (*1*) or 18 percent (*2*) reduction in enzyme activity when taken for in vitro analysis. This is not usually enough reduction for physiological in-

hibition (*4*); on the other hand, it is an unsatisfactory way to investigate inhibition, because drug and enzyme are analyzed together, in vitro, which dilutes and distorts the original relation (*5*).

Keller (*6*) observed inhibition of carbonic anhydrase from bovine red cells by DDT (50 μg/ml) in vitro, whereas Wistrand [cited in (*4*)] found no inhibition of bovine enzyme, in amounts claimed by Keller. Anderson and March (*7*) were unable to detect any inhibition by DDT on insect carbonic anhydrase either in vivo or in vitro at concentrations up to 3550 μg/ml. We examined again, in vitro, whether DDT inhibits this enzyme. The matter is of much theoretical and practical importance, since carbonic anhydrase inhibitors clearly reduce the rate of calcium deposition in shell, both in birds and invertebrates [reviewed in (*4*)].

Carbonic anhydrase activity was analyzed by a colorimetric *p*H method (*8*) which measures the rate of hydration of carbon dioxide. Solutions of *p,p'*-DDT and *p,p'*-DDE were prepared in absolute ethanol or in DMF (*3*). The final concentration of DDT or DDE in the reaction vessel was 50 to 85 μg/ml in 16 percent ethanol or 5 percent DMF. Solutions were incubated with enzyme (human red cell) and solvent (with and without drug) up to 3 days at room temperature. No inhibition was observed. Concentrations greater than 50 μg/ml in the reaction mixture resulted in some precipitation of drug.

The effect of DDT on semipurified bovine carbonic anhydrase was examined by the method of Maetz (*9*), which had also been used by Keller (see above). This method measures the rate of dehydration of carbonic acid. A number of solvents were used; DMF (2.5 percent in final solution) yielded the most reliable data. Again Keller could not be confirmed; there was no inhibition at 100 μg/ml. Inhibition progressed from 37 to 88 percent as the concentration of DDT increased from 500 to 2000 μg/ml. In these experiments there was also some precipitation of drug in the reaction mixture. However, the degree of inhibition observed at 500 μg/ml is relatively small and suggests that DDT may not inhibit carbonic anhydrase effectively at the usual tissue concentrations found in organisms exposed to DDT in the environment. Further clarification is required because of solubility difficulties in the in vitro system. In addition, direct investigation of the inhibition of enzyme from the shell gland of birds is needed. However, no case has yet arisen in tissues of the vertebrate kingdom in which carbonic anhydrase inhibition by known drugs has been greater than that found against the enzyme in red cells (*4*).

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Tumor Immunity: Tumor Suppression in vivo Initiated by Soluble Products of Specifically Stimulated Lymphocytes

Abstract. *Supernatant fluids of specifically stimulated lymphocyte cultures were purified. Fractions containing migration inhibition factor when injected intradermally into strain-2 guinea pigs produced a reaction similar in appearance to delayed cutaneous hypersensitivity. There was an accumulation of mononuclear cells at the injection sites and the growth of syngeneic tumor grafts at the sites was suppressed.*

The immunologic rejection of tumors in syngeneic animals is mediated by specifically sensitized lymphoid cells (*1*). Delayed hypersensitivity has been associated with the rejection of some syngeneic hepatomas induced by diethylnitrosamine (*2*). However, hepatomas that do not provoke a delayed hypersensitivity reaction can be inhibited at the site of a delayed hypersensitivity reaction initiated by an unrelated antigen. Delayed hypersensitivity reactions consist of the specific recognition of an antigen by a relatively small number of sensitized lymphocytes followed by the accumulation of a relatively large number of mononuclear cells (*3*). We have found that macrophages from unimmunized animals, but not neutrophils or lymphocytes, can inhibit the growth of one of these tumors in vivo and in vitro (*4*). Cell-mediated tumor immunity, therefore, requires at least two distinct reactions: (i) specific interaction of sensitized lymphocytes and tumor cell antigen, and (ii) the local accumulation of mononuclear cells that prevent the growth of tumor cells at that site.

Lymphocytes incubated in vitro with the specific antigen to which they were sensitized produce substances that (i) inhibit the migration of macrophages from capillary tubes (*5*), (ii) are cytotoxic in vitro (*6*), (iii) are leukotactic (*7*), and (iv) can give skin reactions similar to delayed hypersensitivity (*8*). Tumor cell antigens have been shown to cause the release of macrophage migration inhibition factor (MIF) (*9*). We have been able to obtain inhibition of tumor growth at sites of inflammatory reactions produced by the intradermal injections of crude supernatants of specifically stimulated lymphocyte cultures (*10*). In this report we show that intradermal injection of tissue culture fluids containing MIF is followed by the accumulation of mononuclear cells and an inflammatory response at the site of injection. The growth of tumors at these sites is inhibited.

Age-matched, adult, Sewall-Wright NIH inbred strain-2 guinea pigs were used. Induction of primary hepatomas by the administration of diethylnitrosamine in the drinking water and the formation of an ascites variant have been described (*11*). Ascites cells from the sixth generation of a transplantable hepatoma (line 10) were prepared (*12*). In all experiments 10^6 tumor cells mixed with the appropriate reagent were injected intradermally in a volume of 0.1 ml. Each result given is the mean for three animals.

Inbred strain-2 guinea pigs were immunized by the injection of heat-killed *Mycobacterium tuberculosis* (0.1 ml,