Induction of Immunologic Tolerance to Human Transferrin and Subsequent Antibody Response to Hemopexin Containing Transferrin.* (30429)

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Although transferrin can be purified from human serum relatively free of hemopexin, it has not been possible to obtain hemopexin free of transferrin. Injection of rabbits with hemopexin preparations containing small amounts of transferrin results in a substantial production of antibody to transferrin. To develop a quantitative method for hemopexin determination, to follow purification of this protein during the isolation procedures, and to conduct clinical studies it was important to obtain large amounts of antibody to hemopexin that was free of antibody to transferrin. Absorption of the antisera with transferrin was not desirable since it is difficult to remove efficiently all the antibody to transferrin without drastically reducing the smaller amount of hemopexin antibody. Therefore, another method for obtaining rabbit antibody to hemopexin was investigated.

It appeared that immunologic tolerance induced in rabbits could be employed to produce an antiserum to hemopexin present in a mixture of 2 or even more proteins. Immunologic tolerance to a number of serum protein antigens has been induced in rabbits by neonatal injection of the proteins(1). The tolerant state is specific and persists for at least 6 months after the rabbits reach maturity(2). In the present study, rabbits were rendered tolerant to transferrin by neonatal injection of transferrin. When these rabbits became mature, they were tested for tolerance to transferrin and then immunized with a preparation containing hemopexin and the contaminating transferrin.

Materials and methods. Antigens: Purified transferrin was supplied by Behringwerke, Inc., Marburg, Germany. Hemopexin was purified from Cohn fraction IV-7 (Cutter Laboratories), according to the method of Schultze *et al*(3). Hemopexin prepared in this fashion still contained substantial amounts of transferrin and an unidentified α_2-globulin. In addition, hemopexin which contained only trace amounts of transferrin was kindly supplied by Behringwerke, Inc. Proteins were trace-labeled with I^{131} by a modification(4) of the method of Hunter and Greenwood(5), and protein-bound activity was determined in a well-type scintillation counter containing a NaI crystal.

Induction of tolerance. Ten albino rabbits from 2 litters each received a total of 70 mg transferrin in 10 subcutaneous injections given every other day starting on the day of birth. At an age of 84-87 days they weighed 2.5-2.9 kg, and were injected with 10.7 mg of I^{131}-labeled transferrin. Simultaneously 5 control animals of approximately the same weight (2.3-2.7 kg) and age received the same dose. The rabbits were bled at various intervals, and the elimination of the I^{131}-labeled protein from the blood was analyzed graphically.

Immunization. At 112-115 days of age, 7 of the tolerant and 4 control rabbits were injected each week for 3 weeks with complete Freund's adjuvant containing hemopexin. Each rabbit received a total of 32 mg of protein. Sixteen days after the last injection, the

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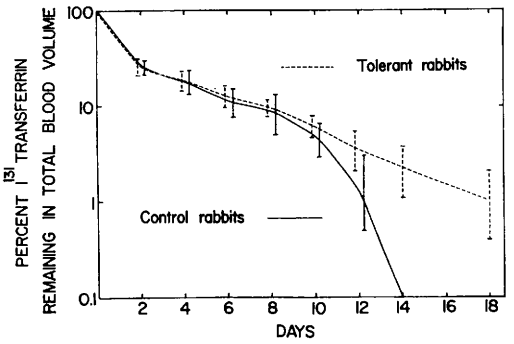

FIG. 1. Elimination of I^{131}-labeled transferrin from blood of transferrin-tolerant rabbits and control animals.

rabbits were bled. One tolerant animal received 2 injections, one week apart, of 5 mg of the purified hemopexin supplied by Behringwerke, Inc. This animal was bled 23 days after the last injection.

The presence or absence of antibody to transferrin and hemopexin (Behringwerke) was determined by the Preer "double diffusion in agar" technique(6). The sera also were analyzed for anti transferrin by a quantitative precipitin test employing I^{131} transferrin(7).

Results. Upon reaching maturity 8 of the 9 surviving rabbits injected neonatally with transferrin failed to show an immune elimination of an intravenous injection of I^{131} transferrin (Fig. 1) indicating that a tolerant state to transferrin had been induced. Following equilibration between intra- and extravascular spaces, the I^{131} transferrin was eliminated at an exponential rate with a half-life of 5.2 days. On the other hand, 5 control, nontolerant rabbits and one rabbit injected neonatally with transferrin after equilibration and an initial exponential elimination of the injected I^{131} transferrin showed an immune elimination, which was complete by the 14th day.

Four control rabbits injected with Freund's adjuvant containing hemopexin (prepared by us) produced precipitating antibody to hemopexin and also to the transferrin contaminant as detected by double diffusion in agar. The level of precipitating antibody to transferrin was 66.6, 119.7, 105.6, and 104.3 μg antibody N/ml of sera. Seven tolerant rabbits injected similarly produced precipitating antibody to hemopexin, but not to transferrin. In addition 3 of the tolerant and all 4 non-tolerant animals produced precipitating antibody to the unidentified α_2-globulin. The one tolerant rabbit injected with Behringwerke hemopexin also formed precipitating antibody to hemopexin but not to transferrin. A normal control animal injected with the same preparation formed antibodies to both hemopexin and transferrin. No appreciable difference in antibody levels to hemopexin was noted between sera of control and tolerant rabbits as indicated by the time required for formation of precipitin bands in double diffusion in agar.

Discussion. Acquired immunological tolerance to transferrin can be induced in rabbits with relative ease, by neonatal injections of transferrin. The above results are similar to those reported in the induction of tolerance to serum albumin and serum gamma globulins(1). A number of properties of transferrin are probably responsible for the ease with which tolerance to it can be induced in the rabbit. *First,* transferrin rapidly equilibrates between intra- and extravascular spaces, which permits it to come in contact with all of the potential antibody producing cells. *Second,* this protein persists in the body fluids and is eliminated only slowly at an exponential rate. *Third,* it is a relatively simple antigen as compared to microbial and tissue antigens.

As with immunologic tolerance to other serum protein antigens, tolerance to transferrin is specific and injection of the rabbits tolerant to transferrin with a mixture of hemopexin and transferrin results in an antibody response to only hemopexin. In the above studies antisera to hemopexin, free of anti-transferrin, were obtained following injection of rabbits with hemopexin containing transferrin as a contaminant. The use of immunologic tolerance to exclude the production of a specific antibody is probably applicable to other protein mixtures in which the contaminating protein can be obtained in a relatively pure form.

Summary. Immunologic tolerance was readily induced in rabbits to human transferrin by neonatal injection of transferrin. In-

jection of the tolerant rabbits, at maturity, with a mixture of hemopexin and transferrin resulted in production of antibody to hemopexin but not to transferrin. Injection of non-tolerant rabbits with the same mixture resulted in production of antibody to both antigens.

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Depression of Whole Blood Folate Activity by Colchicine.* (30430)

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It is well established that colchicine exerts a beneficial effect in the treatment of gouty arthritis in humans, and is capable of producing mitotic arrest in a wide variety of plant and animal cells, but the manner in which these effects are produced is obscure (1,2). Although gouty arthritis in humans is characterized by increased quantities of uric acid in blood and tissues, it has not been possible to demonstrate any alteration of purine metabolism during colchicine therapy. Colchicine has been classified as a "spindle-poison" in contrast to the "chromosome poisons," such as nitrogen mustard and urethane(2). There has not been, to the best of our knowledge, any explanation of colchicine effect in biochemical terms at the molecular level. Recent studies have shown that small doses of colchicine produce abnormal intestinal absorption of D-xylose without detectable alteration in histologic pattern by routine microscopic examination(4). Histochemical techniques on the other hand have revealed prompt and striking reduction in the activity of several oxidative enzymes and co-factors in the mucosal cells of the small intestine(5). Because of the important role of folic acid in the production of cells by the intestinal epithelium and other active tissues, the following investigations have been carried out to explore possible relationships between colchicine and folic acid metabolism.

Materials and methods. Microbiological assays of folic acid activity were carried out by standard methods utilizing *Lactobacillus casei* (ATCC 7469), and commercially available culture medium based on the formula of Baker *et al*(6). Determinations were performed on whole blood. To release the various folic acid derivatives from their conjugated form in red cells, the following procedure was employed, based on the method of Toennies and Phillips(7). Two ml of heparinized whole blood were mixed with 2 ml of 0.05 *N* ammonium hydroxide and allowed to stand at room temperature for 60 minutes. One-half ml of this hemolyzed solution was diluted to 25 ml with freshly prepared 0.5% potassium ascorbate in .05 *M* phosphate buffer, giving a 1 to 100 dilution, and incubated at 37°C for 90 minutes. Conjugase activity was destroyed by autoclaving for 2½ minutes at 15 p.s.i., after which samples were cooled and filtered through Whatman #1 filter paper. Aliquots of 1, 2 and 4 ml were pipetted into culture tubes, 1 ml of the potassium ascorbate buffer solution was added to each tube and the volume brought to 5 ml with distilled water. Five ml of assay medi-

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