Influence of Deuteration on Circulating Antibody Levels in the Mouse.* (31120)

BENJAMIN V. SIEGEL AND JANE I. MORTON
Department of Pathology, University of Oregon Medical School, Portland

The isotopic substitution of deuterium for hydrogen has been shown to markedly alter the relative rates of chemical reactions and to affect the conformation and stability of macromolecules(1). The profound physiological and biological changes associated with high degrees of deuteration have been found not to be compatible with survival in the mammalian organism(2). However, the continuous ingestion with 30% deuterium oxide (D_2O, heavy water) appears to have no appreciable effects on the body weights, general well-being or life span of the laboratory mouse(3). We have recently demonstrated the protective effects of this, and lower levels of D_2O on mice infected with the Rauscher leukemogenic virus(4). The mechanism of protection appeared to be depression of the neoplastic proliferation of lymphoreticular cells. In view of similarities noted between cells undergoing leukemic proliferation and those responding immunologically(5) it seemed feasible to investigate the effects of deuteration on the immune response.

Materials and methods. Deuterium oxide (supplied as 99.7% D_2O by the Richland Operations Office of the U. S. Atomic Energy Commission, Richland, Wash.) was purified by distillation from alkaline permanganate. BALB/c/jax female mice, 5-7 weeks old, were fed a standard pellet diet and deuterium was administered as drinking water *ad libitum* at a concentration (v/v) of 30% for the duration of the experiments.

Mice received a single intraperitoneal injection of 1.25 mg bovine serum albumin (BSA, crystalline, Pentex, Inc., Kankakee, Ill.) in 0.25 ml of a complete Freund's adjuvant emulsion (Difco Laboratories, Detroit) either 10 days or 17 days after they started drinking heavy water. Sheep erythrocytes were washed 3 times in 20 volumes of cold saline and the packed cells suspended in 2 volumes of saline for intraperitoneal injection either as a single dose of 0.25 ml or as two 0.25 ml inoculations 18 hours apart. Mice were bled periodically from the retro-orbital plexus. Antibody titers to BSA were determined by the agglutination of tanned, antigen-coated sheep erythrocytes as described by Stavitsky(6). Red cell agglutinins were determined directly in a 2-fold serial dilution system in the presence of 1:200 saline diluted normal rabbit serum. To assay macroglobulin agglutinin activity, aliquots of mouse antisera were incubated for 18 hours at 25°C in phosphate buffered saline, pH 7.4, containing 0.1 M 2-mercaptoethanol(7). Serial dilutions and assays were carried out directly in this medium. Statistical significance of data was determined by student t-test analysis of reciprocal logarithmic (base 2) titer values. Both BSA-adjuvant and sheep erythrocyte immunization experiments were repeated twice using 5-8 mice in each group with similar results as reported below.

Results. The antibody response of mice drinking 30% D_2O is illustrated in Fig. 1. Mice drinking heavy water continuously starting 10 days in advance of immunization showed no significant titer variations from the controls at the 5% level. Mice that had been drinking 30% D_2O for 17 days before immunization, however, showed depressions of antibody through the 25th day that were significant at the 1% level. At 38 and 50 days, similar titers were achieved for both groups, levels of antibody in the deuterated mice having been restored to control values. Ninety days following the initial BSA-adjuvant immunization, control and deuterium-treated mice received 2 intraperitoneal injections of sheep erythrocytes 18 hours apart. Six days later, mean logarithmic titers were 10.7 for the control animals and 7.3 for the deuterated mice, demonstrating a significant depression ($P<0.001$) in the response of the deuterated animals to this second antigen.

* Supported by U. S. Atomic Energy Commission Contract RLO-1927-3.

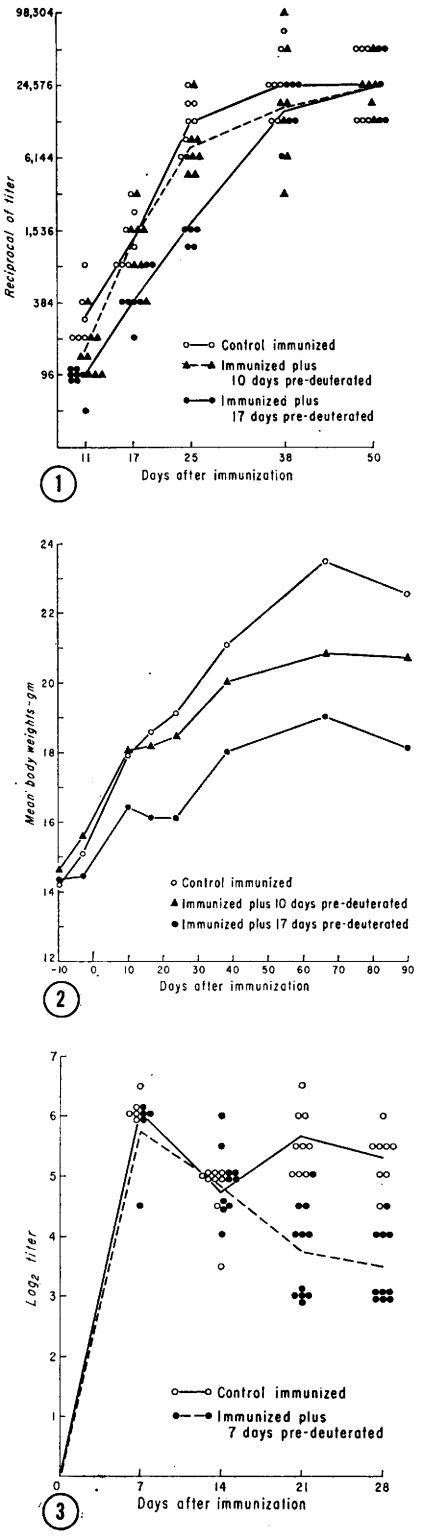

FIG. 1. Response of mice to immunization with BSA in complete Freund's adjuvant. Control-immunized mice and deuterium-treated mice immunized after 10 and 17 days pre-deuteration are compared. Points represent individual animals.

FIG. 2. Mean body weights of mice immunized with BSA in complete Freund's adjuvant. Weights of control-immunized mice are compared to those of mice started on deuterium oxide 10 days and 17 days prior to immunization.

FIG. 3. Agglutinin response to sheep erythrocytes of control mice and mice treated with 30% D_2O starting 7 days prior to immunization. Points represent individual animals.

Body weights of these mice were also followed and were observed (Fig. 2) to be lower in animals drinking heavy water. These differences appeared to be directly related to the length of time the mice had been predeuterated and to the associated effect on the immune response.

Immunization of normal mice with a single injection of sheep erythrocytes resulted in a bimodal response (Fig. 3). In mice similarly immunized after 7 days of deuteration, no depression of titers was observed in the first mode, suppression becoming manifest, however, in the second phase. When sera derived from control mice immunized with sheep cells were subjected to mercaptoethanol treatment, there was a loss of activity of 75% noted at 7 days post-immunization, 50% at 14 days, and unchanged agglutinin titers thereafter. Sheep cell titers for both normal and deuterated animals were found to be less than 1:8 prior to immunization.

Discussion. The restoration of circulating antibody to control levels after 38 days in the mouse drinking heavy water is reminiscent of the net recovery evidenced in immunized animals subjected to certain dose levels of chronic irradiation(8). This phenomenon might be ascribed to a delay in attainment of peak titer resulting from a slowing down of synthetic processes. Alternatively, one might postulate the increase in activity of a select, deuterium-resistant clone of cells producing antibody to BSA. This possibility could be elucidated by examination of the relative distribution of antibody activity among the globulin fractions during the course of deuteration(8). Recovery does not appear to be due to a general restoration of the mouse's immunological capacities in the presence of continuing deuteration, since the pri-

mary response to a second antigen was seen to be diminished in these same animals.

The extent of deuteration at the time of immune stimulus appeared to govern the subsequent antibody response. After 7-10 days pre-deuteration with 30% D_2O, there was little or no depression of the immune response, although by this time equilibrium deuteration of the body fluids to 22-24 atoms per cent would have been reached(3). The data suggest that not until extensive incorporation of deuterium into non-exchangeable positions of cellular structural elements (after 17-22 days deuteration) was there marked effect on the circulating antibody level. In this regard, Katz and his co-workers(3) demonstrated that the incorporation of deuterium into non-exchangeable sites of spleen, liver and kidney tissues was dependent upon synthesis and did not become stabilized until after 3 weeks. Solvent effects and the effects of the attained level of deuterium substitution at exchangeable molecular sites, then, would not appear to be a significant determinant in the observed suppression of antibody.

Drinking 30% D_2O has been observed not to bring about a growth retardation or weight loss in young adult mice during the first 2-3 months of treatment(3,4). However, it was noted in the present experiments that body weights of adjuvant-immunized mice on heavy water were lower than those of immunized animals drinking H_2O. These differences could be ascribed to the marked proliferation of tissues of the reticuloendothelial system (RES) that occurs in the adjuvant-injected mouse, resulting in an increase in body weight (9). In the case of immunized animals on D_2O, this weight increase was less marked. Thus, a large part of the antibody suppressive effects observed in the D_2O study employing adjuvant immunization may have been due to inhibition of RES cell proliferation.

On the other hand, deuterium-depressed RES function at the time of antigen administration would not appear to account for the suppression of hemagglutinins in mice inoculated only with sheep cells, where diminished titers were not evident until some time after 14 days post-immunization. The extent of deuteration achieved at time of immunization and for some days thereafter was not sufficient, evidently, to depress levels of early antibody. The bimodal agglutinin response, here, on immunization with sheep cells, with a predominance of mercaptoethanol sensitive antibody in the first peak is comparable to that described by Adler(10) for CBA mice similarly immunized with sheep erythrocytes. This specific diminution of later antibody by deuterium may, therefore, be associated with a direct effect on the 7S antibody producing cell or its precursors.

Summary. Depressed serum antibody levels were observed in mice drinking 30% D_2O continuously, starting 17 days before immunization with BSA in complete Freund's adjuvant. Adjuvant-induced increases in body weight were also diminished in these animals. Ten days pretreatment with heavy water did not, however, alter the response to BSA. In another group of mice pretreated with heavy water for 7 days, primary agglutinin levels to sheep red cells were normal in the continuously deuterated mice for the first 2 weeks following immunization, diminishing thereafter. The extent of heavy water pretreatment required suggests that mere equilibration of body fluids and exchangeable tissue hydrogens with 30% D_2O does not result in depressed antibody levels. Only after substantial deuterium was incorporated by synthesis into non-exchangeable sites were circulating antibody titers observed to be diminished.

1. Flaumenhaft, E., Bose, S., Crespi, H. L., Katz, J. J., Int. Rev. Cytol., 1965, v18, 313.

2. Thomson, J. F., Biological Effects of Deuterium, Pergamon Press, New York, 1963, p85.

3. Katz, J. J., Crespi, H. L., Czajka, D. M., Finkel, A. J., Am. J. Physiol., 1962, v203, 907.

4. Siegel, B. V., Life Sci., 1964, v3, 261.

5. Dameshek, W., Ann. N. Y. Acad. Sci., 1965, v124, 6.

6. Stavitsky, A. B., J. Immunol., 1954, v72, 260.

7. Deutsch, H. F., Morton, J. I., Science, 1957, v125, 600.

8. Draper, L. R., in The Effects of Ionizing Radiation on Immune Processes, C. A. Leone, Ed., Gordon & Beach, New York, 1962, p221.

9. Morton, J. I., Siegel, B. V., Vox Sang., 1966, in press.

10. Adler, F. L., J. Immunol., 1965, v95, 26.

Received February 14, 1966. P.S.E.B.M., 1966, v122.