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Deuterium Isotope Effects on Lymphoid Tissues and Humoral Antibody Responses in Mice*

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Summary. Hematological and other morphological changes following body deuteration were evaluated in young adult pathogen-free female Swiss albino mice given heavy water (99.8% D_2O) as drinking fluid. Control groups included mice given tap water ad libitum, distilled water, or amounts of tap water equivalent to the reduced fluid intake of animals drinking D_2O. Deuterated mice manifested changes in their spontaneous activity, weight loss, fall in body temperature, atactic gait, ruffled fur, decrease in breathing rate and preterminal cachexia. The mice were killed in a moribund state after exposure to D_2O for about 2 weeks. At the time of death, packed red blood cell volumes were within normal range. Mean erythrocyte counts were not markedly reduced. In contrast, there was a conspicuous blood lymphocytopenia. The bone marrow displayed a normal cellularity and a moderate reduction in the proportion of erythropoietic cells. A particularly striking weight loss of thymus and spleen, and a marked systemic lymphocyte depletion were apparent, particularly in the thymus, white pulp of the spleen, lymph nodes, and gut-associated lymphoid tissue. Signs of increased cell death were found in lymphoid organs. The number of mitotic figures in intestinal cryt epithelia was reduced. Since D_2O is known to interfere with DNA synthesis and mitosis, the deuterium-induced systemic lymphocyte depletion was probably due to disturbed cell proliferation and increased cell death.

On the basis of these data, the effects of reduced levels of deuteration on antibody production and thymic morphology were also evaluated. Adult female Swiss albino mice, raised under specific pathogen-free conditions, were given drinking fluids composed of 10%, 20% and 40% D_2O during the experimental test period. After 7 days exposure, all animals were given a primary subcutaneous injection of tetanus toxoid, followed by a booster

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injection 49 days after primary stimulation. All animals were killed 7 days after the booster. Deuteration resulted in a dose-dependent depression of specific antibody production. Primary antitoxin responses were more affected than secondary responses. A marked reduction of the thymic cortical area of deuterated mice was proportional to the dose of D_2O. Deuterium-induced immunosuppression is tentatively linked to a diminution of the total body lymphoid cell mass and suppression of the plasmocytoid cells involved in antibody synthesis.

Key words: Deuterium oxide – Lymphoid tissue – Cell division – Immunosuppression – Antibody formation.

Introduction

In 1932, Urey, Brickwedde and Murphy reported the isolation of deuterium (^2H, D). This heavy stable isotope of mass 2 occurs in natural water in the order of 1 part in 6,700 parts of ^1H. Deuterium has been used for nuclear fusion reactions; it constitutes the explosive of the hydrogen bomb in the form of lithium deuteride (Windholz et al., 1976). If controlled thermonuclear fusion can be harnessed for energy production, deuterium is likely to become increasingly important. Currently, biochemical and biophysical studies often have recourse to this stable isotope. The very low cross section for neutron capture makes it useful, in the form of heavy water, as a moderator in nuclear reactors.

Even before the preparation of pure heavy water (D_2O), Lewis (1933) predicted that it might interfere with chemical reactions in living organisms to such an extent that life would be threatened. This prediction was found to be true in a host of experimental situations. Mice, rats and dogs do not survive more than 35% of D_2O substitution for H_2O in their body fluids for long periods of time (reviews: Thomson, 1963; Katz and Crespi, 1970; Finkel, 1973). In contrast, algae, bacteria, yeasts, molds and a few protozoans can be grown in higher concentrations of deuterated water, and complete substitution of ^1H for ^2H can be achieved in some of these lower organisms.

The information on organ and tissue changes induced by heavy water in mammals is fragmentary. There are even less data available on the effects of D_2O on immune systems in mammals. Stoner (1978) has recently demonstrated a singular suppressive action of D_2O on antibody formation following primary and secondary stimulation of mice with tetanus toxoid.

The present study describes marked atrophy of lymphoid tissues in mice exposed to various doses of heavy water, and relates structural changes to the deuterium-induced suppression of primary and secondary tetanus antitoxin responses.

Materials and Methods

Two sets of experiments were done. The objective of the first set (I) was an evaluation of hematological and histopathological variables in a relatively small number of mice given virtually pure heavy

water to elicit maximum effects. The information thus obtained served as a baseline for the second experimental set (II) focussing on the effect of moderate body deuteration on antibody production and thymic morphology.

I. Subacute Toxicity of D_2O

This experiment was done in duplicate. One set included the following four groups of specific pathogen-free female Swiss albino mice (Füllinsdorf strain) 8 to 12 week old:

1. "D_2O"-Group. The freely accessible drinking fluid in overhead bottles consisted of D_2O with 99.8% deuterium (Eidgenössisches Institut für Reaktorforschung, Würenlingen, Switzerland). Tritium contamination of this batch of heavy water was determined by counting D_2O samples in a liquid scintillation counter (TriCarb B 2450, Packard Instruments) and was found to be at least 10 times less than the maximum permissible concentration in water for occupational exposure in man (NCRP, 1959; SSVO, 1976).

2. "Tap Water, Restricted"-Group. Since animals have been observed to resist deuteration by drinking less (Thomson, 1963), suitable controls were included for the effects of restricted water intake such as dehydration and stress. This was done by giving to control mice a daily volume of drinking fluid – tap water – corresponding to the volume of heavy water used on the previous day by the same number of mice in the D_2O group.

3. "Tap water" was given ad libitum to mice of this group.

4. "Distilled water", ad libitum.

The animals of each group comprising five mice in the first experiment and six in the second were kept in a cage of the shoebox type provided with wood bedding (Weichholzgranulat Typ TE 1/2, Gabriel Schill AG, Muttenz, Switzerland) and given standard mouse chow (Mäuse- und Rattenzucht Alleinfutter Nr. 850, Nafag, Gossau, Switzerland). The deuterated mice were killed by an ether overdose when moribund, as judged by weight and activity loss, poor condition and decrease in breathing rate. At autopsy, white and red blood cell counts, packed red cell volume, blood and bone marrow smears were obtained. Heart, lung, kidney, adrenal, spleen, thymus, parathymic, axillar, bicipital, cervical, mesenteric, lumbar, caudal and popliteal lymph nodes, salivary glands, esophagus, stomach, small intestine with all Peyer's patches, large intestine, liver, pancreas, sternum, lumbar spine, femur, pituitary gland, and brain were removed. Large organs were weighed. All organs were processed for conventional histology. Undecalcified bone was embedded in methacrylate.

II. Effects of D_2O on Antibody Responses and Thymic Morphology

The experimental conditions are summarized in Table 1. Female Swiss albine mice of the Hale-Stoner strain, raised under specific pathogen-free conditions, were 3.5 to 4 month old at the start of the experiment. Heavy water was obtained from Brookhaven National Laboratory stock, 99.86%. Only trace amounts of tritium activity in the D_2O were detected as described in section I. The various percentage solutions were prepared on a volume/volume basis with tap water.

Fluid tetanus toxoid (FTT) and *aluminum phosphate-adsorbed tetanus toxoid* (APTT) were obtained from Lederle Laboratories (Pearl River, New York). Secondary responses were elicited 49 days after primary stimulation. Pooled blood for serum was obtained from the caudal artery (0.2 ml) on day 14, 28 and 42. All animals were killed 56 days after primary stimulation (7 days after secondary) by an overdose of ether. Pooled sera were prepared from each group for titration as a single specimen. *Serum antitoxin titers* were determined in test mice by neutralization of potent tetanus toxin. Each serum was titrated 3 times. Minimal paralysis at the end of 4 days was used as the end point (Hale and Stoner, 1956). The possibility that the presence of deuterium in the serum might interfere with the combining capacity of specific antibody for tetanus toxin was considered. Sera diluted with 50% and 100% D_2O were titrated along with similar saline

Table 1. Effects of D_2O on antibody responses and thymic morphology: experimental conditions

Drinking fluid[a]	Primary stimulation[b]		Secondary stimulation[c]
	FTT	APTT	
Tap water	13	13	26
10% D_2O	14	14	28
20% D_2O	14	14	28
40% D_2O	13	11	24
Total number of mice			106

[a] Presented in overhead bottles equipped with ball-valved stainless steel drinking tubes, beginning 7 days before primary immunization, on day −7

[b] Single subcutaneous injection of either 0.2 ml fluid tetanus toxoid (FTT) or of 0.05 ml aluminum phosphate-adsorbed toxoid (APTT) on day 0

[c] Single subcutaneous injection of 0.1 ml FTT in all animals on day 49

controls, and were also used in precipitin tests. Since there was no difference in the combining or precipitating capacity of the sera diluted with D_2O, it was assumed that the physico-chemical properties of D_2O did not contribute to the reduced titers of antitoxin observed in mice maintained on D_2O (Stoner, 1978).

The thymus was carefully dissected at autopsy, weighed, and processed for conventional histology. The cross-sectional area and the area occupied by the cortex was determined in at least 4 transverse thymic sections.

Results

I. Subacute Toxicity of D_2O

Experimental values obtained from animals given tap water or distilled water did not differ significantly.

The mean daily fluid consumption is shown in Fig. 1. These values include fluid actually consumed and fluid lost by the animals toying with the bottle tip, bottle handling and evaporation. The amount of D_2O consumed per day was roughly half of that fluid volume used by control mice given tap or distilled water ad libitum. The mean food consumption was slightly less in deuterated mice compared to control animals, i.e. 3.5 g/mouse/day, versus 4.1 g/mouse/day in the group with restricted water intake, and 4.5 g/mouse/day in the two other groups. Whereas the control animals gained weight during the experimental period, mice given D_2O or similarly restricted amounts of tap water lost weight (Fig. 1). This loss was consistent and more severe in deuterated animals. On day 11, a slight increase in D_2O consumption, accompanied by a slight transient gain in body weight was noted.

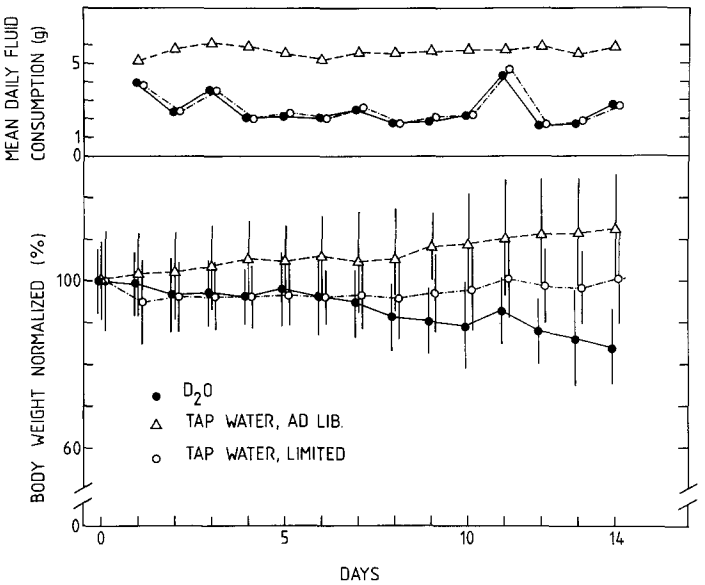


Fig. 1. *Top:* Daily drinking fluid consumption versus time. Mice in the "tap water restricted" – group were given amounts of tap water equivalent to the reduced intake of deuterated counterparts. *Bottom:* Mean body weight versus time

A diurnal restlessness of deuterated mice became apparent early in the experiment. An aggressive behaviour, although expected from earlier descriptions (Bachner et al., 1964), was not seen in experimental mice. Some animals jumped convulsively on their hind legs, along the cage wall, at the start of the second week of deuteration. These episodes occurred at lengthening intervals and later disappeared. We did not notice epileptoid seizures. Around day 14, the gait became broader and hesitant. The reactions of the mice to handling were reduced. The fur was ruffled, and body temperatures were lower in deuterated animals. A marked fall in breathing rate to about half of the control value, severe weight loss and cachexia were taken as signs of impending death. The majority of the mice was killed at the end of the second week of deuteration.

At autopsy, a small volume of dark, red-brown blood was drawn from the heart, approximately half that of control mice with unrestricted water intake. The urinary bladder was empty. The intestines usually contained small amounts of food. The organs, particularly liver and spleen, appeared to be paler than control organs. The adrenals were tan.

Hematological findings are summarized in Figs. 2 and 3. Packed red blood cell volumes of deuterated mice were within normal range. Mean erythrocyte counts were slightly reduced (Fig. 2). Conversely, there was a marked lymphopenia in deuterated mice, whereas white blood cell counts of mice given restricted volumes of tap water were in the normal range (Fig. 3). Deuterated organs were reduced in weight as compared to control animals, with the exception of the brain (Fig. 4). Organ weights were also slightly reduced in mice with

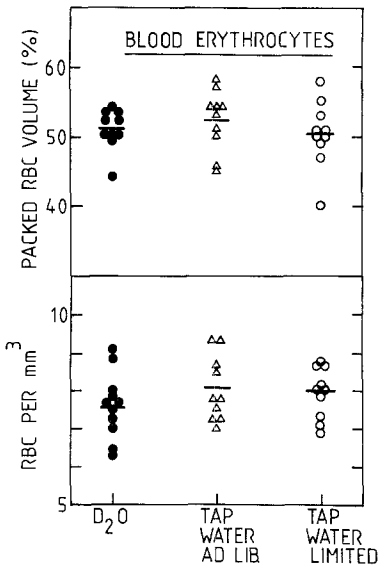


Fig. 2. Packed red blood cell volume (*top panel*) and red blood cell counts (*bottom panel*) in the various groups. Each full or open circle corresponds to one animal. Horizontal bars indicate the mean

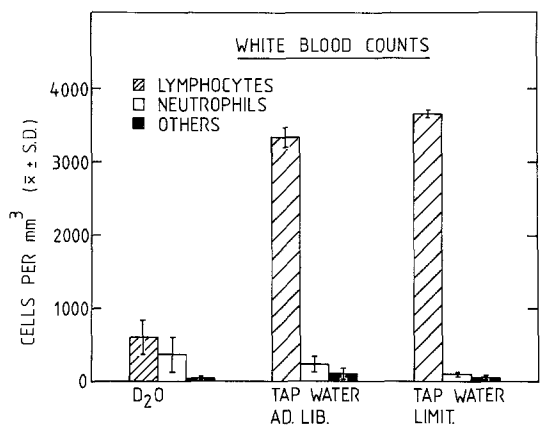


Fig. 3. White blood counts at the time of death. Note marked lymphocytopenia of deuterated mice

restricted tap water intake, most likely because of dehydration. A particularly striking weight loss of thymus and spleen, however, was noted only in deuterated mice. Histological examination of the thymus of these animals revealed a narrow, hypocellular cortex surrounding a relatively dark medulla with moderate numbers of small lymphocytes (Fig. 5). There was no comparable thymic atrophy in animals with similarly restricted intake of tap water. The cross-sectional surface area of the thymic cortex of deuterated mice was about 2/5 of the total thymic area, compared with a value of 4/5 in the control groups (Fig. 6).

The splenic white pulp of deuterated animals contained very few small lymphocytes compared with controls (Fig. 7). Germinal centers were virtually absent. There were a large number of tingible bodies in the white pulp, and reticulum cell-like elements predominated in a wide marginal zone. The red

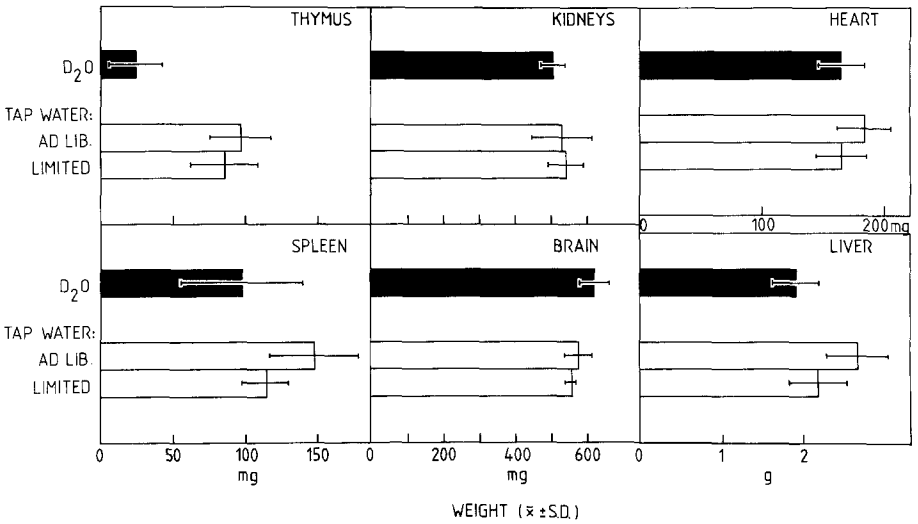


Fig. 4. Mean organ weights. Note marked reduction of thymus and spleen weight of deuterated mice. The weight of the brain in deuterated animals slightly exceeds that of control mice with restricted tap water intake

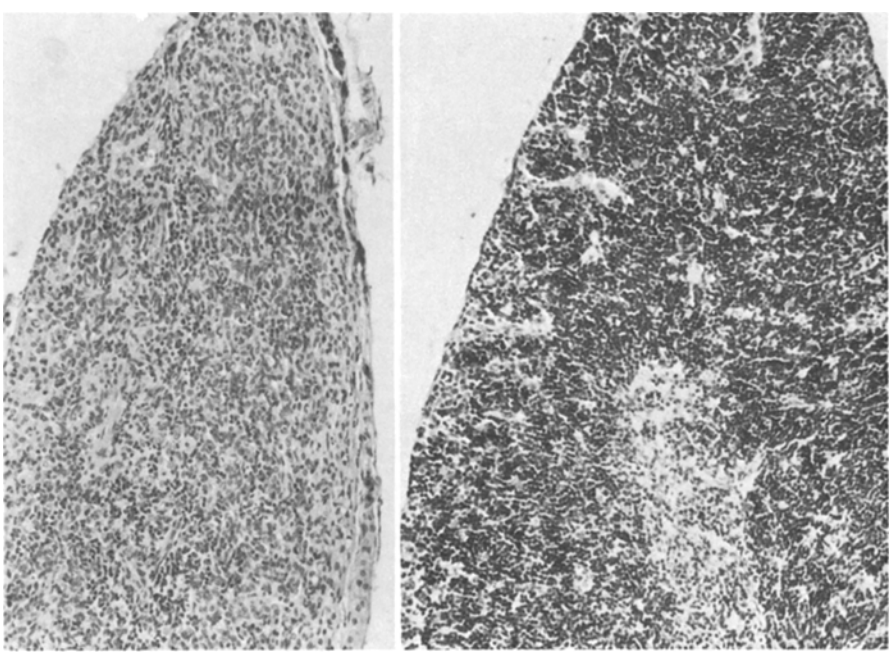


Fig. 5. Thymus, H&E, × 125. *Left:* deuterated mouse. *Right:* mouse with restricted tap water intake. The hypocellular outer zone at the left contrasts with a wide, dark, cellular cortex of the control organ

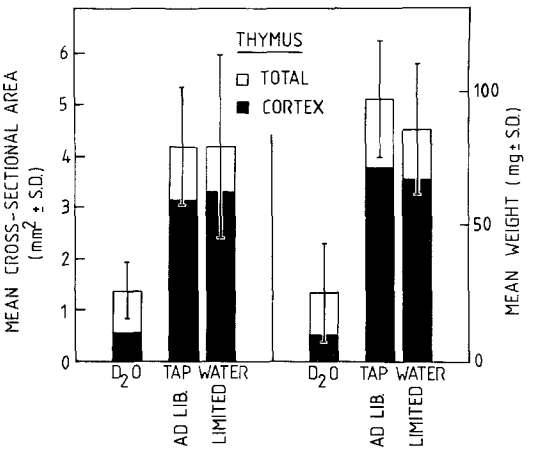


Fig. 6. Mean thymus and thymus cortex cross-sectional area (*left*), and mean thymus and thymus cortex weight (*right*). Note significant reduction of both parameters in deuterated mice

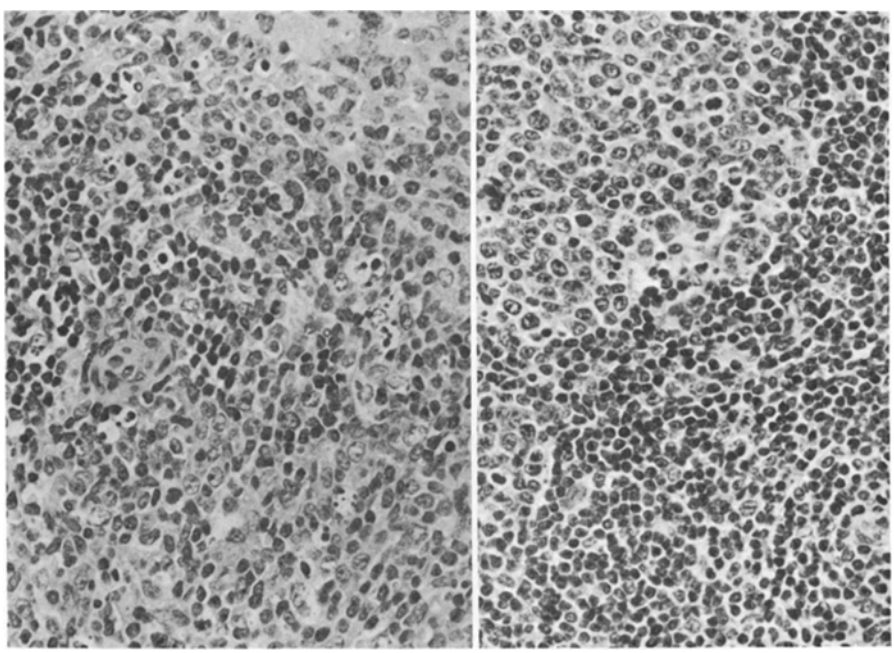


Fig. 7. Spleen, H&E, ×300. *Left:* deuterated mouse; a small, hypocellular periarteriolar lymphoid zone. Large numbers of nuclear debris. Wide marginal zone. *Right:* control mouse with restricted tap water intake. Note the large follicle with germinal center (*top*) and densely packed small lymphocytes

pulp appeared hypocellular, with marked hemosiderosis. The spleen of water-restricted controls displayed moderate amounts of intracellular iron-positive pigment. Few hemopoietic cells were seen in deuterated mice. The lymph nodes of experimental animals were often hypocellular, particularly lacking small lymphocytes (Fig. 8). Germinal centers were rare and small.

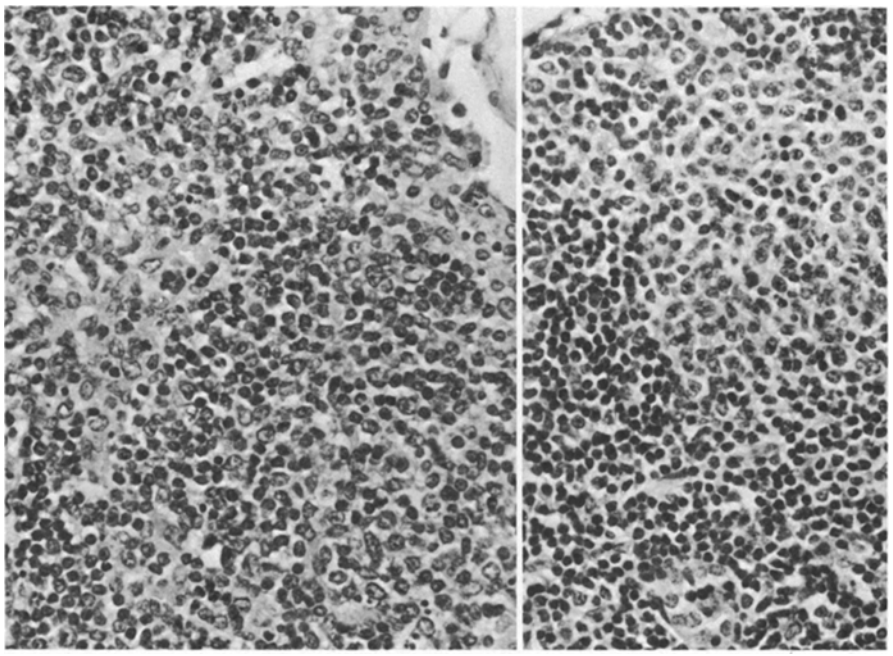


Fig. 8. Lymph node, H&E, ×300. *Left:* small node of a deuterated animal. Hypocellular cortex. Dearth of primary and secondary follicles. *Right:* normal aspect. Mouse with restricted tap water intake. Lymphatic follicle with germinal center. Cellular paracortex

Peyer's patches of deuterated mice were small, hypocellular and devoid of germinal centers. Similar changes were not seen in animals with restricted tap water intake.

Bone sections revealed a normal cellularity of the marrow (Fig. 9). In smears and in sections, a moderate reduction in the proportion of erythropoietic cells was observed in most of the deuterated mice. Otherwise, major differences in bone marrow structure were not apparent. Bone marrow hemosiderosis was slight in both experimental and control groups.

The small intestinal lumen of deuterated mice was often narrow, and the villi appeared somewhat shorter than those of controls. The mitotic index of crypt epithelia was lower than observed in mice with restricted water intake (Fig. 10).

There were surprisingly few conspicuous histological changes in all the other organs examined. The myocardium revealed a variable staining intensity and a cloudy swelling of the muscle fibers. Parenchymatous organs such as liver, kidneys, pancreas and salivary glands mainly displayed a slightly increased granularity of the cytoplasm. The brain of deuterated mice showed slight edematous changes and minor alterations in chromatin structure of some neurons in cortical areas and cerebellum. A detailed neuropathological analysis of central nervous tissues is in progress.

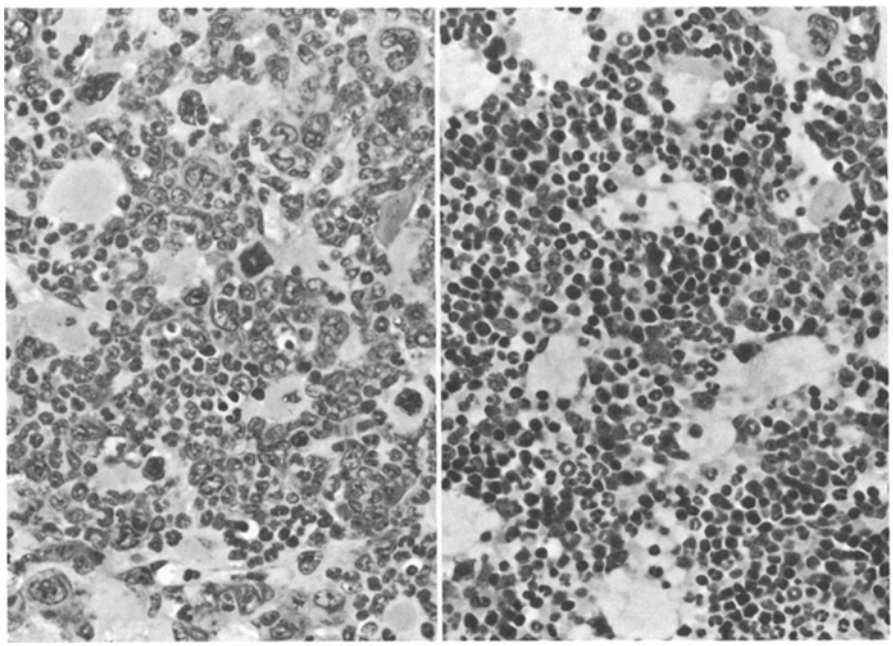


Fig. 9. Bone marrow, methacrylate section, Giemsa, ×300. *Left:* deuterated mouse. Normal cellularity. Reduction in the proportion of erythropoietic cells. *Right:* control mouse with restricted water intake. Several erythropoietic foci

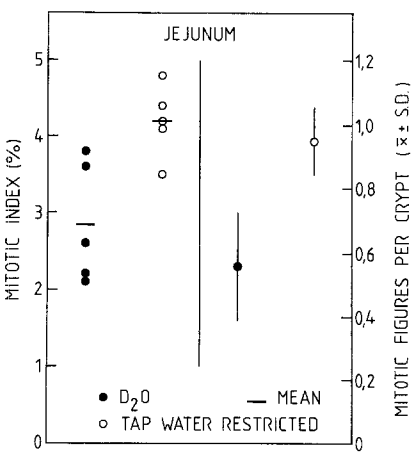


Fig. 10. Deuterium-induced depression of the mitotic indices of jejunal crypt epithelia (*left panel*; the full and open circles represent individual animals; the horizontal bar indicates the mean). *Right panel:* number of epithelial mitotic figures per jejunal crypt

II. Effects of D_2O on Antibody Responses and Thymic Morphology

Antibody titration results are summarized in Figure 11. There was a dose-dependent depression of primary responses in deuterated animals. Low concentrations of D_2O in the drinking water (20%) resulted in responses reduced

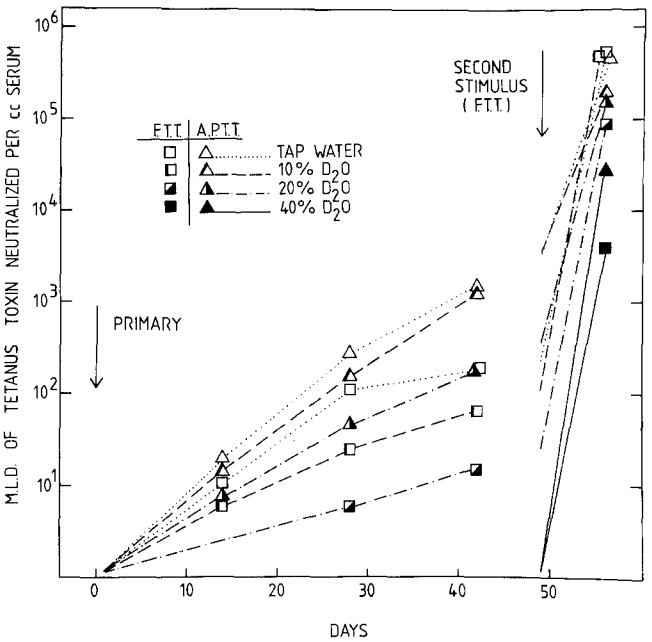
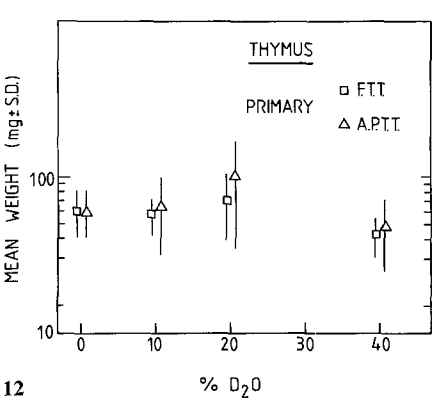


Fig. 11. Serum antibody levels versus time following primary stimulation on day 0 and secondary stimulation on day 49. Deuterated water was given from day – 7 throughout the experiment. There is a dose-dependent degression of antibody formation in deuterated mice, particularly of primary responses. There were no detectable antitoxin levels following primary stimulation of mice given 40% D_2O. Priming with aluminum phosphate-adsorbed toxoid (APTT) results in higher antibody titers as compared to a fluid tetanus toxoid (FTT)



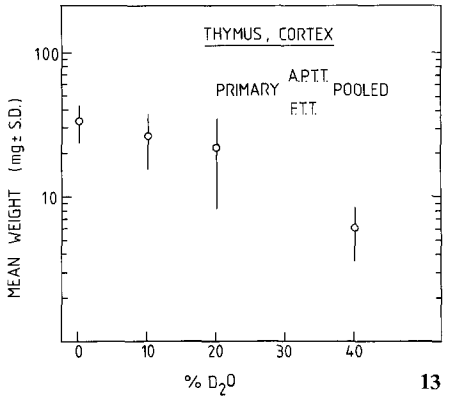


Fig. 12. Mean thymic weight on day 56 after primary stimulation and 7 days after booster injection, versus concentration of D_2O in the drinking fluid. Animals exposed to 40% D_2O display a marked thymic weight loss. Thymic weights of animals primed with FTT or APTT do not differ significantly

Fig. 13. Mean weight of the thymic cortex on day 56 versus concentration of D_2O in the drinking fluid. Semilogarithmic plot. There is an inverse, near-linear relationship between thymic cortex weight and concentration of D_2O in the drinking fluid

to approximately 10% of control values on day 42. The suppression of APTT-induced primary responses was less apparent. Secondary responses were also depressed in deuterated mice, in a dose-dependent pattern. Antibody titers of mice maintained on 40% D_2O were one to two orders of magnitude below control values. Antibody titers in experimental and control animals were lower following primary stimulation with FTT as compared to those with APTT, particularly during the primary response.

The thymic weight was within control ranges in mice given 10% or 20% D_2O, and reduced in mice given 40% D_2O (Fig. 12). The thymic cortical area, however, was clearly reduced in deuterated mice (Fig. 13). The semilogarithmic plot reveals a near-linear inverse relationship between thymic cortical area and concentration of D_2O in the drinking fluid.

Discussion

Ingested heavy water is readily absorbed by the gastrointestinal tract and equilibrates with both extra- and intracellular body fluids (Thomson, 1963). It appears that there is no selective urinary excretion of D_2O. Equilibrium deuteration in the body fluid of mice is reached around the 10th day after introducing deuterated water in various concentrations (Katz et al., 1962). In the present study, the serum levels of deuterium were not determined. The ratio of D_2O concentrations in serum and drinking fluid is dose-dependent and known to lie between 0.6 and 0.8 on the 10th day of deuteration (Katz et al., 1962; Laissue and Slatkin, 1976). A full equilibration with the drinking fluid is never achieved because food (Thomson, 1963) and water exchanged with inhaled air (Pinson and Langham, 1957) provide significant amounts of non-deuterated water. The rate and magnitude of serum deuteration is not markedly different from corresponding serum tritiation patterns in rodents drinking tritiated water (Thomson and Ballou, 1956). The deuterium contents of non-aqueous components reaches equilibrium by the third week, approaching one-half that measured in the water distilled from the tissues (Kath et al., 1962).

Water is ubiquitous in mammals. It is therefore not surprising that substitution of ^1H for ^2H with a change in mass ratio by a factor of 2 (the largest for any pair of stable isotopes of the same element) should induce solvent and constitutional isotope effects. Ionic equilibria in D_2O differ significantly from those in H_2O (Katz and Crespi, 1970). The smaller self-ionization of D_2O versus H_2O makes pD different from pH (Covington et al., 1968). The higher melting (3.81° C) and boiling point (101.42° C), the higher density and viscosity, and the poorer solvent properties for salts and gases compared to H_2O result in a modification of the fluid environment in the body and may thus affect biochemical reactions (Katz and Crespi, 1970). Furthermore, constitutional isotope effects arising in organic molecules through replacement of ^1H by ^2H probably affects the stability of hydrogen bonds (Calvin et al., 1959; Scheraga, 1960), in particular of biopolymers such as proteins and DNA (Alexandrov et al., 1965).

Substitution of more than 35% of body water for D_2O is not compatible

with mammalian life. Median survival times for mice deuterated to higher levels range from 60 to 10 days for mice drinking 40%, 75% (Katz et al., 1962) or 100% (Häggqvist and v. Hevesy, 1956) D_2O, respectively. Animals drinking 30% D_2O have a normal life span (Katz et al., 1962). Strain differences and specific pathogen-free conditions may also play an important role, since the median survival time of our mice maintained on 99.8% D_2O clearly exceeded 10 days.

The symptoms noted here in the course of deuteration are quite similar to those mentioned in earlier reports (Hansen and Rustung, 1935). The effects of a restricted fluid intake, as monitored in the group of mice given limited fluid volumes, appeared to be negligible compared to the conspicuous changes due to deuterium intoxication. The reason for the slight increase in D_2O consumption on day 11 is unknown. The changes in activity have been related to deuterium-induced disturbances of biological rhythm. The length of the period of mouse circadian activity rhythm is directly proportional to the concentration of D_2O in the drinking fluid (Dowse and Palmer, 1972). Altered diffusion and ionic flux rates following body water deuteration have been considered among the possible pathogenetic factors. Deuterium-induced decreases in metabolic rates, and a progressive loss in body temperatures of mice have been described in detail by Barbour and Trace (1936).

The most conspicuous changes in morphology following subacute deuterium intoxication consisted of the marked depletion of lymphoid tissues – thymus, spleen, lymph nodes, gut-associated lymphoid tissues – and in blood lymphopenia. Earlier reports mention an atrophy of splenic lymph follicles in mice with body water deuterated to less than 30% within 2 weeks (Dybing et al., 1938). Thymic changes, systemic lymphocyte depletion, and lack of germinal centers in lymph nodes and Peyer's patches have not been reported. The marked blood lymphopenia is in good agreement with the observations of Czajka et al. (1961). These investigators also described a relative lymphopenia and a progressive anemia in dogs maintained on 50% D_2O as drinking fluid for more than 10 days. A marked leukopenia has been observed in mice drinking water deuterated to 50% for 12 to 16 days (Gustavson and Häggqvist, 1957); differential counts, however, were not markedly changed, and anemia was slight.

Decreased lymphocyte production and increased lymphoid cell death results in lymphopenia. Histological signs of cell disintegration were clearly visible in spleen and lymph nodes of our animals. The shrinking of the thymic cortex, in excess of the reduction in total thymic weight, is in keeping with disturbed thymocyte proliferation. Earlier investigations have revealed a depressed thymic uptake of ^{125}I-Iododeoxyuridine, a synthetic DNA precursor, in mice drinking 15% or 30% D_2O (Laissue and Slatkin, 1976). These findings point to a reduced proliferative activity of the thymus.

Packed red blood cell volumes were within normal range in mice drinking D_2O. Mean erythrocyte counts were slightly reduced. This is in keeping with earlier studies in mice (Gustavson and Häggqvist, 1957). Thomson (1960) witnessed a progressive anemia and an increase in reticulocyte counts in rats after prolonged exposure to drinking water deuterated to 50%. Exposure to D_2O for a period in excess of ten days is apparently required to elicit anemic changes in rodents. While bone marrow cellularity appeared normal in our mice, a relative diminution in the number of erythroid precursors was noted in deuter-

ated animals. We have not found data on bone marrow examination in other studies. Since the mean life span of mouse erythrocytes is about 40 days (Russell and Bernstein, 1968), the effects of a reduced medullary and probably splenic erythropoiesis would not be apparent in the experimental period of 14 days in the absence of significant destruction of erythrocytes. The livers and spleens of deuterated mice appeared to be paler than control organs at autopsy.

The interference of deuterium with DNA synthesis has also been observed in the bone marrow. Maurer and Wenzel (1977) have shown a depressed uptake of tritiated thymidine by mouse bone marrow cells in vitro following preincubation with D_2O. A specific DNA activity of 40% of that of control values was measured by Wilson and Dinning (1961) following incubation of rabbit bone marrow cells in a medium made up with 85% D_2O and ^{14}C-thymidine. Administration to rabbits of a drinking fluid containing 50% D_2O for 30 days resulted in a potent inhibition of DNA synthesis in bone marrow and thymus, as expressed by the inhibition of radiophosphate incorporation into DNA (Dicken et al., 1962). In the present experiment, a test of the subacute toxicity of D_2O, panmyelocytopenia was not seen. In a previous study, ^{125}I-Iododeoxyuridine incorporation in the bone marrow of mice was found to remain relatively unaffected by moderate body deuteration for 3 weeks (Laissue and Slatkin, 1976). More prolonged exposure to D_2O using sublethal doses may be required to elicit obvious and measurable bone marrow depletion.

Depressed mitotic indices in the small intestinal crypt epithelia of deuterated mice indicate an interference of deuterium with cell division. Extensive experimentation, mainly on the development of fertilized eggs, has established the antimitotic activity of deuterium (Marsland and Zimmermann, 1963, 1965; Gross and Spindel, 1960). Mitosis of sea urchin eggs can be held "frozen" as long as the deuterium remains in the culture medium. Mitotic abnormalities have been observed in cell renewal systems of deuterated animals, particularly in the testes (Häggqvist and v. Hevesy, 1956; Amarose and Czajka, 1962).

The minor pathological changes in the other organs of deuterated animals are in good agreement with earlier findings (Dybing et al., 1938; Czajka et al., 1961; Bachner et al., 1964). Some deuterium-induced alterations such as degradation of endoplasmic structures of renal tubular epithelia and salivary gland are evident only after ultrastructural analysis (Zunker and McKay, 1966). The sum of the morphologic findings failed to give a satisfactory explanation of why mammals die from deuteration. Nor does the evidence for impairment of renal, cardiovascular and central nervous system function point to a particular target organ (Finkel, 1973). There is obviously a widespread interference with a host of metabolic processes following body deuteration.

The results of the second series of experiments clearly demonstrate the inhibitory effects of heavy water on antibody formation to tetanus toxoid. Primary responses are more effectively suppressed than secondary responses. Higher amounts of D_2O, at least 40% in the drinking water in this experiment, are required to inhibit secondary responses significantly. Our observations are in keeping with the slight decrease in precipitating antibody reported in mice maintained on 30% D_2O for 17 days prior to immunization with bovine serum albumin, described by Siegel and Morton (1966). Enhanced primary responses

following stimulation with toxoid absorbed to aluminum phosphate, versus fluid toxoid, are well known. The phenomenon is probably related to temporal differences in antigen concentrations and a slow release of antigen to lymphoreticular organs (Cottier et al., 1970).

One of the principles governing immune systems is the activation of few committed precursor cells, their proliferation and differentiation, resulting in a larger population of functional effector cells. Thus, cell proliferation is intimately tied to the ability to respond to an antigenic stimulus. Virtually all of the antibody-forming cells appearing in a primary response are the direct progeny of cells which were dividing at the initial period of the response (Syeklocha et al., 1966; Dutton and Mishell, 1967). In vivo studies using suppression of humoral responses by radiation have led to the same conclusions (Grobler et al., 1974). Antibody responses are profoundly depressed when whole body X-irradiation preceeds primary injection of FTT by as little as one hour. Higher doses of radiation are required to effect depression of secondary responses to the level of primary responses (Stoner and Hale, 1962). Six hundred and fifty to 800 rads of whole body radiation markedly depress secondary antitoxin responses when delivered shortly before, or up to 3 days after, injection of the booster. In contrast, whole body irradiation of mice 4 days after the booster does not reduce plasma cell formation appreciably in the medulla of lymph nodes regional to the site of antigenic stimulation, nor does it affect antibody production, even though most small lymphocytes and germinal centers are rapidly destroyed (Grobler et al., 1974). Experiments combining autoradiography and immunofluorescence have shown that virtually all plasma cells containing specific antibody after secondary antigenic stimulation have originated from precursors which had entered DNA synthesis and presumably mitosis after the antigenic stimulus (Baney et al., 1962). The results of the irradiation experiments, therefore, indicate that antibody production was mainly due to radioresistant plasma cells, i.e. the progeny of cells proliferating during the first 3 days after the booster (Grobler et al., 1974). Since both ionizing radiation and heavy water interfere with cell proliferation, similar patterns of immunosuppression may be expected after comparable temporal exposure to the damaging agent. The level of deuteration in the present experiment was high enough to result in disturbed cell proliferation. Full equilibrium deuteration of the mice was reached at the time of the secondary, but not after primary stimulation. Based on earlier studies, however, the ratio of D_2O concentrations in serum and drinking fluid must have been approximately 0.5 on the day of primary stimulation (Katz et al., 1962; Laissue and Slatkin, 1976). This would correspond to a serum level of about 20% D_2O in the highest dose group. Marked effects on lymphoid cell proliferation have been observed even below this serum concentration (Laissue and Slatkin, 1976).

The *immunosuppressive effect* of ionizing radiation is primarily due to damage to *small lymphocytes* and *proliferating immunocompetent cells* (Grobler et al., 1974). The relationship between the deuterium-induced, dose-dependent loss of cortical thymocytes, and suppression of humoral antibody responses indicates the possibility of analogous mechanisms in deuterated animals. The thymus is unquestionably a major site of lymphocyte production (Laissue et al., 1976).

Thymus-derived cells constitute the majority of recirculating lymphocytes. Previous studies have indicated that primary antibody responses to tetanus toxoid were initiated by both circulating and sessile lymphocytes (Stoner et al., 1969). Peripheral, differentiated, thymus-derived T-lymphocytes express helper and suppressor activity during antibody production. In rodents, this population may not develop fully following either neonatal thymectomy or adult thymectomy combined with whole-body irradiation (Miller and Osoba, 1967). The ability of Swiss albino mice to respond to antigenic stimulation is only partially impaired by neonatal thymectomy (Hess et al., 1963). Deuterium-induced damage to cortical thymocytes could thus have affected the production of thymus-derived lymphocytes instrumental in humoral immune responses, resulting in deficient antibody production. The loss of cortical thymocytes seen in the subacute toxicity experiment can be taken as an indicator of systemic lymphocyte depletion also involving B cells and is presumably of greater importance. Therefore, it appears reasonable to assume that the deuterium-induced suppression of the humoral immune response to tetanus toxoid was brought about at least in part by a diminution of the body lymphoid cell mass, together with inhibition of cellular proliferation of the lymphoplasmocytoid cells involved in antibody production.

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