incompatible with either mechanism of operation of this organ.

Summary. 1. The capacity of spleen cells from mice thymectomized during neonatal life to elicit graft *vs* host reactions was studied using Simonsen's graft *vs* host assay of histocompatibility. 2. The reaction of A strain spleen cells from mice sham operated or thymectomized in the neonatal period was compared to that of isologous F1 cells in (A × C57Bl)F1 recipients and Z strain spleen cells from similarly treated groups were compared in (Z × C57Bl)F1 and in the (Z × DBA/2)F1 hybrids. 3. The results show that early complete thymectomy in both A and Z mice renders the spleen cells of these animals incapable of producing graft *vs* host reactions in F1 hybrids. 4. These data are interpreted as evidence that the cells themselves of the lympho-reticular system in mice thymectomized at or shortly after birth are immunologically defective. 5. These observations are considered as a further evidence that the thymus plays a key role in development of immunological capacity in mammals.

1. Good, R. A., *Bull. Univ. Minn. Hosp. and Minn. Med. Foundation,* 1954, v26, 1.

2. MacLean, L. B., Zak, S. J., Varco, R. L., Good, R. A., *Surgery,* 1956, v40, 1010.

3. Gafni, J., Michaeli, D., Heller, H., *New England J. Med.,* 1960, v263, 536.

4. Fichtelius, K. E., Laurell, G., Philipson, L., *Acta Path. et Microbiol. Scand.,* 1961, v51, 81.

5. Archer, O., Pierce, J. C., *Fed. Proc.,* 1961, v20, 26.

6. Archer, O., Pierce, J. C., Papermaster, B. W., Good, R. A., *Nature,* 1962, in press.

7. Miller, J. F., *Lancet,* 1961, v2, 748.

8. Martinez, C., Kersey, J., Papermaster, B. W., Good, R. A., PROC. SOC. EXP. BIOL. AND MED., 1962, v109, 193.

9. Martinez, C., Dalmasso, A., Good, R. A., *Nature,* 1962, in press.

10. Glick, B., Chang, T. S., Jaap, R. G., *Poultry Sci.,* 1956, v35, 224.

11. Mueller, A. P., Wolfe, H. R., McGibson, W. H. J., *J. Immunol.,* 1959, v83, 507.

12. Mueller, A. P., Wolfe, H. R., Meyer, R. K., *ibid.,* 1960, v85, 172.

13. Papermaster, B. W., Friedman, D. I., Good, R. A., *Fed. Proc.,* 1961, v20, 36.

14. Papermaster, B. W., Bradley, S. G., Watson, D. W., Good, R. A., *J. Exp. Med.,* 1962, in press.

15. Papermaster, B. W., Friedman, D. I., Good, R. A., PROC. SOC. EXP. BIOL. AND MED., 1962, in press.

16. Papermaster, B. W., Dalmasso, A. P., Martinez, C., Good, R. A., PROC. SOC. EXP. BIOL. AND MED., 1962, in press.

17. Simonsen, M., *Ann. N. Y. Acad. Sci.,* 1958, v73, 834.

18. Simonsen, M., Jensen, E., in *Biological Problems of Grafting.* Ed., F. Albert and G. Lejeune-Ledant, 1959.

Received March 30, 1962. P.S.E.B.M., 1962, v110.

Inhibition of P^{32} Incorporation into Rabbit DNA *in vivo* by Deuterium Oxide.* (27467)

CHARLES DICKEN, THOMAS R. HENDERSON AND JAMES S. DINNING
Biochemistry Department, School of Medicine, University of Arkansas, Little Rock

Animals which have been given deuterium drinking water show widespread effects: impairment of kidney function, anemia, disturbed carbohydrate metabolism, central nervous system disturbances, altered adrenal function and, when approximately one-third of their body water has been replaced by D_2O, death(1). Incubation of rabbit bone marrow cells *in vitro* with deuterium oxide has been reported drastically to inhibit the synthesis of DNA in these cells(2). Moreover, it has been suggested that many of the biological effects of D_2O may be due to alteration of the ordered helix-random coil equilibrium in DNA secondary structure(3).

In view of these findings, we have investigated the effects of deuterium on DNA synthesis *in vivo,* and the effects of deuteration

*This study was supported by research grant from Nat. Inst. Health.

on the physical characteristics of the DNA isolated from representative tissues.

Materials and methods. Four New Zealand rabbits of mixed sexes weighing approximately 300 g, were utilized in this experiment. All of these animals were given Niemeyer's F-84 Fortified Rabbit Chow *ad libitum*. Two animals were placed on 50% D_2O (obtained from Isotopes Specialties Co., Burbank, Cal.) and 2 on regular water. The amount of 50% D_2O was regulated by manual administration with a pipette at intervals during the day. For the first week, the 2 animals on regular water were allowed water *ad libitum*. After the first week, administration and amount were the same for both groups. The animals were given 25 cc of 50% D_2O daily for 17 days. This was then changed to 35 cc of 30% D_2O for the remainder of the experiment.

After 17 days, the first pair (one control and one deuterated animal), were injected intravenously with P^{32} (500 μc/kg), and sacrificed 2 hours later. After 27 days the second pair was injected intraperitoneally and sacrificed. DNA was isolated by the method of Kirby(4). RNA was removed by digestion with RNAase (obtained from Worthington Biochemical Corp., Freehold, N. J.). Samples were counted in an end-window Geiger counter. DNA concentration was measured by the indole method of Ceriotti(5).

DNA heat denaturation profiles were carried out by heating solutions (ca. 10γ/ml of DNA) in 0.15 M NaCl—0.015 M sodium citrate stepwise in a thermostatically controlled water bath with 15-minute equilibration at each temperature and reading the absorbance at 260 mμ in a Beckman DU spectrophotometer.

The hemoglobin, hematocrit, RBC, WBC and reticulocyte count and the Schilling differential count were done with venous blood from the ear vein. Both marrow smears were made from the tibia at the time the animals were sacrificed.

Results. The 2 animals on D_2O showed a progressive decrease in hemoglobin, hematocrit, RBC and WBC counts. The reticulocyte count in the deuterated animals showed a slight increase over the controls. The peripheral blood smears and bone marrow smears did not show significant changes. Fig. 1 and 2 show the values for the animal on D_2O for the longer period. The other deuterated animal exhibited a similar, but less severe, hematological picture at time of sacrifice.

The specific activity of the DNA preparations from the deuterated animals is shown in Table I. The uptake of P^{32} into DNA is thus seen to have been greatly repressed in the bone marrow and in the thymus deuterated animals; incorporation into liver DNA is affected somewhat less.

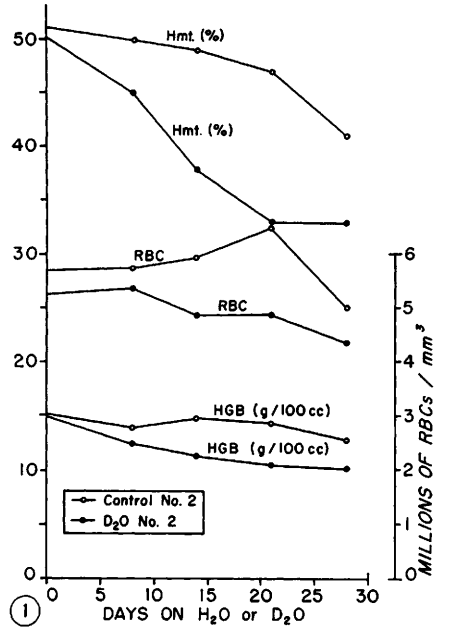
The heat denaturation profiles for the

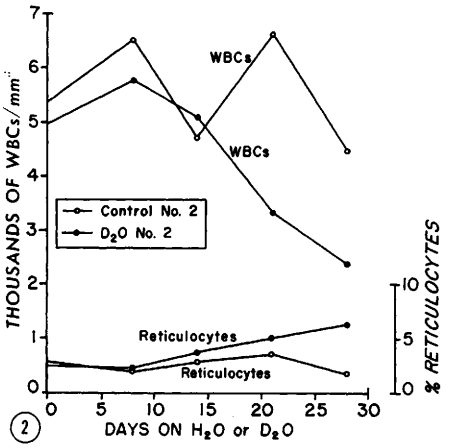
TABLE I. Characteristics of DNA Isolated from Control and Deuterated Rabbits.

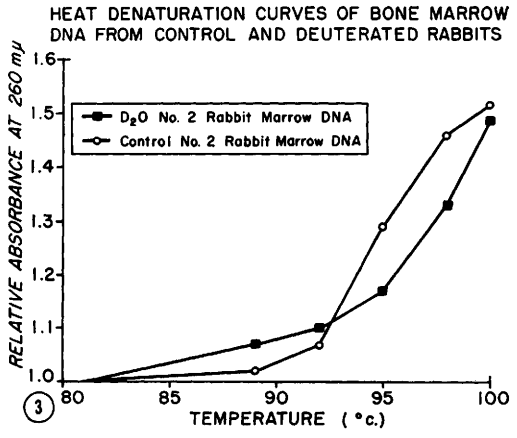
Tissue	Animal No.	Days on 50% D_2O	Days on 30% D_2O	T_m,* °C	% hypochromicity† (260 mμ)	Specific activity, cpm/mg DNA	% inhibition of P^{32} uptake
Marrow	Control 1	0	—	93.8	51	1170	80
	D_2O 1	17	—	95.2	38	232	
	Control 2	0	0	94.7	54	460	97
	D_2O 2	17	11	96.3	50	15	
Thymus	Control 1	0	—	95.1	46	1550	96
	D_2O 1	17	—	94.4	47	56	
	Control 2	0	0	95.2	50	350	98
	D_2O 2	17	11	92.7	46	7	
Liver	Control 1	0	—	93.2	29	108	74
	D_2O 1	17	—	95.0	44	28	
	Control 2	0	0	94.3	48	18	0
	D_2O 2	17	11	94.5	39	21	

* T_m defined as temperature corresponding to midpoint of thermally induced absorbance increase.

† % hypochromicity is % increase in absorbance of a DNA solution after being heated stepwise to 100°C.







DNAs were also found to be somewhat different for the deuterated animals (Table I). Variations in the T_m were found particularly in the case of the thymus and of the marrow. The T_m value has been reported to be one of the most sensitive criteria of the integrity of the DNA molecule(6). Slight differences were noted in the actual heat denaturation—absorption curves (Fig. 3). Similar differences have been noted between DNA preparations from bone marrow cells incubated *in vitro* in a H_2O or 85% D_2O medium respectively(2).

Discussion. The low activity of certain DNA preparations from deuterated animals suggests that inhibition of DNA synthesis by D_2O is a significant effect *in vivo* as well as *in vitro*. The fact that the biological effects of D_2O are most pronounced in tissues with a high mitotic rate suggests that DNA synthesis may be one of the metabolic events most sensitive to D_2O. The possibility exists, however, that the flow of information from DNA to RNA, as well as the flow of information to new DNA, may be inhibited by deuteration. The subtle variations in the heat denaturation profiles of DNAs from deuterated animals, suggest that the incorporation of deuterium into exchangeable and non-exchangeable positions of DNA molecules has modified the secondary structure. This configurational change may be responsible for the observed inhibition of DNA replication. It is entirely possible that even slight variations in structure may interfere with DNA functioning *in vivo*.

The absence of any gross changes in the morphology of the circulating erythrocytes or in the bone marrow itself further suggests that the defect is primarily one of cell replication. The lower activities of the DNA from the 28-day deuterated animals indicate that the inhibition becomes more profound in most tissues as deuteration proceeds. The lower specific activities of the DNA from 28-day controls is probably a reflection of a slower growth rate.

FIG. 1. Effect of D_2O administration on hemoglobin, hematocrit and erythrocyte count in rabbits.

FIG. 2. Effect of D_2O administration on leukocyte count and reticulocyte count in rabbits.

Summary. Administration of 50% deuterium oxide to rabbits as drinking water has been found to result in potent inhibition of DNA synthesis in the bone marrow and thymus, and to a lesser extent, in the liver. A decreased number of circulating erythrocytes was observed under these conditions, but no significant change in erythrocyte or bone marrow morphology was noted. DNA preparations from deuterated animals appear to be slightly denatured in comparison to DNA preparations from control animals.

1. Thomson, J., *Ann. N. Y. Acad. Sci.,* 1960, v84, Art. 16, 736.
2. Wilson, J. C., Dinning, J. S., *Biochim. Biophys. Acta,* 1961, v53, 223.
3. Henderson, T. R., Dinning, J. S., *Nature,* 1962, v194, 498.
4. Kirby, K. S., *Prog. in Exp. Tumor Res.,* 1959, S. Karger Pub., New York.
5. Ceriotti, G., *J. Biol. Chem.* 1955, v214, 59.
6. Marmur, J., Grossman, L., *Proc. Nat. Acad. Sci. U. S.,* 1961, v47, 778.

Received March 26, 1962. P.S.E.B.M., 1962, v110.

Emetic Responses of Monkeys to Apomorphine, Hydergine, Deslanoside and Protoveratrine.* (27468)

M. T. PENG AND S. C. WANG
Dept. of Physiology, College of Medicine, National Taiwan University, Taipei, Taiwan, and Dept. of Pharmacology, College of Physicians and Surgeons, Columbia University, New York City

The discovery of the chemoceptive emetic trigger zone in the area postrema led to reinvestigation of the sites of action of many emetic agents(1). It has been reported that apomorphine(2), morphine and Hydergine (3), and intravenous copper sulfate(4) caused vomiting by acting on the trigger zone; intravenous digitalis glucosides act mainly on the trigger zone but also on other areas(1); and oral copper sulfate acts peripherally on the gastrointestinal tract(4). It has also been found that X-radiation(5,6) and motion sickness(7) induce vomiting by acting on the trigger zone. Most of these experiments have been done in dogs and some in cats(8) and it has been shown that there are a number of differences in emetic mechanism between these 2 species(9,10,11). It is interesting to study, therefore, whether the emetic mechanism in monkeys differs from that in the other 2 animals. Brizzee *et al.*(12) reported that the trigger zone of monkeys is virtually unresponsive to drugs, but the action of various emetic agents in these animals has been investigated far less thoroughly than it has been in dogs and cats.

We chose 3 groups of drugs: first, drugs which cause vomiting by acting solely or mainly on the trigger zone, such as apomorphine(2) and Hydergine(3) and deslanoside (1,8); second, drugs which act on the gastrointestinal tract, such as oral copper sulfate (4); third, drugs which cause emesis by acting on areas other than the trigger zone, such as protoveratrine A(13). Our results indicate that while monkeys are less sensitive to all emetics, both those acting on the trigger zone and those acting on peripheral sites, the trigger zone of these animals is indeed responsive to drugs.

Materials and methods. Fifty-two monkeys (*Macacus cyclopis*) of either sex weighing 2.3 to 5.2 kg were used. Animals receiving emetic agents intravenously such as apomorphine, Hydergine, deslanoside and protoveratrine A† were fed about 20 minutes before injection. Those challenged with copper sulfate were fasted for at least 18 hours before the compound, calculated dry and dissolved in 40 ml of water, was given *via* a stomach tube. Gut denervated monkeys were

* Supported by Grant from Nat. Inst. of Neurol. Dis. and Blindness.

† Hydergine, deslanoside and protoveratrine A were kindly supplied by Sandoz Pharmaceuticals, Hanover, N. J.