tending the incubation period of initial C-F antigen-antibody mixtures in the cold, a greater fixation is obtained. As a result, however, the specificity of the reactions is greatly decreased. Studies are contemplated to determine the specificity of the test employing various incubation temperatures and incubation times.

Brumfield, *et al.*(7) and Jeon(11) have shown that additional quantities of chicken C'1 are required for initiation of the complement fixation reaction when chicken serum is employed. This requirement for additional chicken C'1 has been confirmed for the Rous sarcoma virus-antibody system in trials with 14 chicken serums. In addition, however, an increased requirement for heated Nch was also noted (Table III). The component in heated Nch appears to be a lytic factor, possibly C'3 or one of its components, since it is present in the inactivated negative test serum (serum No. 12159, Table III).

The importance of the order of addition of reagents for obtaining maximum titers has been emphasized by Brumfield, *et al.*(7). With the RSV antigen-turkey and chicken antiserum systems, the sequence of reagent addition did not greatly influence the titers obtained. In fact, slightly higher titers were noted using the standard sequence when Nch or C'1 concentrate was added to guinea pig complements as described under *Materials and methods*.

Summary. A complement fixation test with Rous sarcoma virus is described and some preliminary results reported. Although the sensitivity of the test appears to be somewhat lower than *in vitro* tissue culture neutralization tests, the correlations obtained are quite good.

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Effect of Deuterium Oxide Incorporation of P^{32} into Rat Liver Deoxyribonucleic Acid.* (27798)

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In recent years considerable attention has been given to the physiological and biochemical effects of deuterium in mammals. Besides gross physiological effects such as hyperexcitability, loss of weight, ulceration of the skin, and eventual death, effects on the functions of vital organs and on metabolic processes have been observed(1-3). Experiments both *in vivo* and *in vitro* have shown that substitution of deuterium for hydrogen in various enzyme systems generally results in a reduction of reaction rates. Although this is not always true, it is commonly expected in reactions

* Preliminary experiments were carried out at Argonne National Laboratory when the senior author was a Semester Student in the Associated College of the Midwest program (1961). This work was done in partial fulfillment of the requirements for Honors at Graduation from Lawrence College, and was carried out in part under the auspices of the U. S. Atomic Energy Commission.

involving C-H bond cleavage, since the deuterium bond to carbon is stronger than the hydrogen bond(4).

There is also ample evidence that D_2O is an inhibitor of cell division(5-7), differing from most other mitotic inhibitors in that it can block cell division at any stage of mitosis. Although mitotic inhibition has been attributed largely to solvent effects on macromolecular organization(7), the influence of the kinetic isotope effect cannot be excluded. The present study was undertaken to determine the effect of increasing concentrations of deuterium (up to the lethal level of 30-35%) on incorporation of P^{32} into rat liver DNA *in vivo*.

Since this work was carried out, there has appeared a report on the inhibitory effects of D_2O on the *in vivo* incorporation of P^{32} into DNA of rabbit thymus, bone marrow, and liver(8), complementing an earlier observation from the same laboratory on the inhibition by D_2O of DNA synthesis in bone marrow *in vitro*(9).

Methods. Fourteen Holtzman male rats, 4-5 weeks old, were maintained in individual cages and fed Purina Laboratory Chow *ad libitum* with periodical supplements of lettuce and carrots. Eleven of the animals were given 50% D_2O as drinking water.† The time required for the animals to reach 30% replacement of H_2O by D_2O was approximately 11 days. Experiments were designed so that all animals were the same age at the end of the experiment. Terminal blood samples were taken by cardiac puncture. The plasma obtained from these samples was analyzed spectrophotometrically for deuterium content by a modification of the method of Crespi and Katz(10).

After overnight food deprivation and 3 hours before sacrifice, each animal was lightly anesthetized with ether and injected intraperitoneally with 200 or 400 μc P^{32}/kg body weight, the more heavily deuterated rats receiving the larger quantity. The P^{32} was purchased from Oak Ridge National Laboratory as the monobasic sodium salt. The volume of each injection ranged from 0.5 to 1.0 ml. After 3 hours, the rats were killed by decapitation. The livers were removed and homogenized in 0.25 M sucrose solution, and the nuclei and heavy cell fragments separated by centrifugation at 600 $\times$ g for 10 minutes. The supernatant was discarded and the DNA initially separated by the method of Schmidt and Thannhauser(11). A 0.5-vol aliquot of 0.1 M carrier phosphate was added to dilute the unincorporated P^{32}. Further purification, which included 4 additional precipitations of the final DNA product, was carried out according to the methods of Juni *et al.* (12) and Thomson *et al.*(13). The final DNA precipitate was dissolved in 1 N NaOH and an aliquot was dried uniformly on a stainless steel planchet. Each sample was counted 3 times for 3 minutes each time in a gas-flow counter. Two additional samples of DNA were assayed for total phosphorus(14). The data are reported as relative specific activity, which is defined here as counts/minute/mg phosphorus/0.1 millicurie P^{32}/kg body weight.

Results. The relative specific activity of the DNA is plotted against percent deuterium in the plasma in Fig. 1. A steady decrease in activity is shown by the least squares plot to a point where the incorporation rate of P^{32} is reduced to about one-fifth of that of the controls. The activities reported appear high in comparison with those of Thomson *et al.* (13) and Barnum and Huseby(15). However, the difference probably is a reflection of the differences in the age of the animals, since Fukuda and Sibatani(16) have shown that the mitotic index of the liver of rats of the same age as those used here is about 10 times greater than that of the animals of the ages used in either of the studies cited above(13, 15).

Discussion. The inhibition in rate of incorporation of P^{32} into DNA increases progressively with increasing deuterium concentration, until at the highest level of deuteration there is an 80% reduction. In view of the general effect on enzyme kinetics, this reduction could easily be attributed to a slowdown in the metabolic pathways leading to the synthesis of DNA. Any step involving

†Deuterium oxide was obtained at greater than 99% purity from Argonne Nat. Laboratory under USAEC contract CNET 62/2-5.

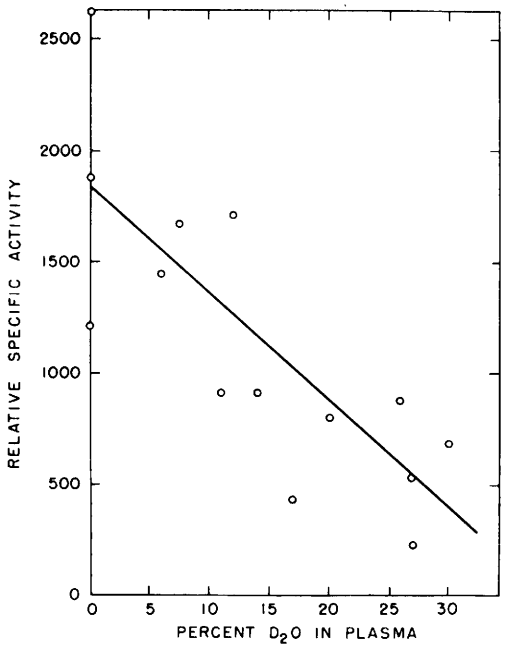

FIG. 1. Effect of increasing plasma D_2O concentration on *in vivo* incorporation of P^{32} into rat liver DNA. The equation of the regression line is RSA = 1821 − 48.1 × %; $\sigma_a = 400$, $\sigma_b = 22.9$.

transfer of a hydrogen atom or some steric-dependent step would be affected.

In their work with *Arbacia* eggs, Gross and Spindel(7) showed that mitosis is inhibited at 30% replacement with D_2O. They considered that this inhibition probably results from a "freezing" of macromolecular structures in which hydrogen-bonded hydrogen has been replaced by deuterium, since the stronger D bonds would tend to slow down the rearrangement of cell structures necessary for division. They proposed further that the kinetic isotope effect did not account for the antimitotic action, since D_2O can inhibit mitosis at stages when duplication of DNA has already been completed. Although the solvent effects of D_2O cannot be ignored, the data presented here demonstrate a progressive inhibition, with increasing deuterium concentration, of DNA synthesis in rat liver.

Dicken *et al.*(8) have shown that incorporation of P^{32} into DNA of rabbit liver is reduced by 74% as a result of administration of 25 ml/day of 50% D_2O for 17 days. Although the age was not specified, their animals were doubtless very young, weighing only 300 g, and were probably growing rapidly; hence it is difficult to estimate the extent of replacement of body water by D_2O. However, the degree of anemia was considerably less than that observed in moribund rats(1). Thus although the magnitude of depression of P^{32} incorporation was about the same in rabbits and rats, a comparison of the results may not be valid.

It may be argued that the depression of P^{32} incorporation is not a direct effect of deuterium but rather is a nonspecific change reflecting the moribund condition of the animals. However, the inhibiting effect of D_2O on P^{32} incorporation into bone marrow DNA *in vitro*(9) indicates that the effects *in vivo* may be specific.

Summary. Experiments were carried out in an attempt to determine the effects of D_2O administration on incorporation of P^{32} into rat liver DNA. With increasing concentrations of D_2O, there was a progressive decrease in DNA synthesis; in rats in which 30% of the body water was replaced by D_2O the incorporation of P^{32} into DNA was reduced to about one-fifth of normal.

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Enhancement of Experimental Allergic Encephalomyelitis by Adrenalectomy.* (27799)

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Corticosteroids or stress suppress, and adrenalectomy enhances, certain immunologic phenomena(1). Experimental allergic encephalomyelitis (EAE) is thought to be due to delayed hypersensitivity to nervous tissue. Corticosteroids(2) or stress(3) are known to suppress EAE. The following experiments show that adrenalectomy enhances development of EAE.

Methods. Female 150-250 g CFN-derived or Sprague-Dawley rats were fed Purina Laboratory Chow and tap water *ad libitum*. Encephalitogenic emulsion was prepared by cycling 40% w/v rat spinal cord homogenate and equal volume of Freund's adjuvant between 2 syringes connected by a double-hubbed needle. Freund's adjuvant (complete) contained 4 mg/ml killed tubercle bacilli suspended in Bayol F-Arlacel A (8.5: 1.5). EAE was produced by administering encephalitogenic emulsion as 7 intradermal injections of 0.05 ml each in plucked skin of flanks and dorsal neck, under light ether anesthesia. Both rat strains were known to be of low susceptibility to EAE when challenged in this fashion(4). Total bilateral adrenalectomy was performed under pentobarbital anesthesia by making a dorsal midline skin incision and longitudinal incisions through muscle and peritoneum on each side of vertebral column; rats with adrenal remnants at necropsy were eliminated. Adrenalectomized rats were maintained on 1% NaCl as sole source of fluid. Adrenal enucleation was performed by snipping off a bit of each adrenal capsule and expressing the glands through the rents; capsules were left *in situ* and subsequently regenerated cortical but usually no medullary tissue. Sham operations were carried to the point of visualizing both adrenals. Except as indicated, each experimental group consisted of 10 rats.

Clinical signs of EAE consisted of paresis or paralysis of one or more limbs and, in severe cases, urinary incontinence. Dragging or limpness of the tail was a common but not completely reliable sign and was not scored. Surviving rats were exsanguinated under chloroform anesthesia 21 days after challenge. The entire spinal cord and hindbrain fixed in Bouin's solution, plus inoculation sites and draining lymph nodes fixed in formalin were embedded in paraffin, sectioned, and stained by hematoxylin-eosin. Histologic evidences of EAE were graded by number of lesions: 1—up to 4 or 5 lesions on the entire slide, 2—a few lesions in each of several different low-power fields, 3—lesions in many different fields, 4—many lesions in all or almost all fields. Slides were read without knowledge of the group from which tissues were derived.

Results. Total adrenalectomy 2 days prior to challenge produced a great increase in incidence and severity of clinical and histologic signs of EAE compared to unoperated controls (Table I). Thymus was much larger and spleen, inoculation sites and draining lymph nodes were slightly larger than controls. Inoculation sites and draining lymph nodes had slightly more epithelioid cell granu-

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