stereospecificity, both with regard to substrate and with regard to coenzyme. A further role of the protein can be envisioned and is illustrated in Fig. 3. Given identical spatial arrangement of the DPN molecules, the presence of a negatively charged grouping on the enzyme would convert a D-lactate specific enzyme into an L-specific enzyme.

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References and Notes

1. Abbreviations: DPN and DPNH, oxidized and reduced diphosphopyridine dinucleotide, respectively; TPN, triphosphopyridine dinucleotide.
2. Contribution No. 226 of the McCollum-Pratt Institute. This work was supported in part by grants from the American Cancer Society, the American Heart Association, and the National Cancer Institute, National Institutes of Health (grant No. 2374C).
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Production of Sterility in Mice by Deuterium Oxide

The present availability of D_2O at a reasonable price has stimulated an increased investigation of its physiological effects. The inhibition of ascites tumor growth and algal reproduction has recently been reported by this laboratory (*1, 2*) and others (*3*).

We have demonstrated the production of sterility in mice by the substitution of D_2O for a part of the drinking water (*4*). In the first experiment, six female and six male C_{57} mice that had been maintained on 30 percent (*5*) D_2O in the drinking water for 4 weeks were mated (*6*). The animals were housed, three females and three males to a cage. Administration of D_2O was continued for 10 weeks. Since there were no pregnancies at the end of this time, the D_2O was discontinued. At the end of another 8 weeks, there still being no pregnancies, three of the treated females were mated with three normal males, and three of the treated males were mated with three normal females. Although the mating of D_2O-treated females with normal males resulted in litters at the end of 3 weeks, all offspring died within 24 hours, and two of the mothers died. The mating of D_2O-treated males with normal females did not produce offspring until the end of 13 weeks, when one female littered. From the three D_2O-treated males and three D_2O-treated females remaining together, one female littered in 4 weeks, one in 10 weeks. This experiment is graphically represented in Fig. 1.

In the second experiment, both C_{57} and Swiss mice were used. A minimum of ten mice of each sex of each strain were maintained on 5, 20, or 30 percent D_2O in the drinking water for 8 weeks, as is indicated in Table 1. At the end of this treatment period, administration of D_2O was discontinued and each mouse was individually mated with a normal mouse of the same strain. At the same time, normal mice of each strain were individually mated as controls. The animals were not handled or disturbed during the first week after parturition, since handling of the young can lead to cannibalism by the parents. Offspring were counted and sexed 2 weeks after birth.

Those pairs which did not produce a litter during a 28-day period after the beginning of mating are considered to be a sterile pair (Table 1). This period allowed 1 week more than the average 21-day gestation period.

Our data, summarized in Table 1, indicate that 30 percent D_2O causes 100 percent sterility (*7*) in both C_{57} and Swiss males. Some of the C_{57} females were also sterile. These data and the unpublished results of another series suggest that 20 percent D_2O in the drinking water for C_{57} males produces almost complete sterility and that 5 percent D_2O in the drinking water of C_{57} mice appears to produce a degree of sterility comparable to that which 20 percent D_2O achieves in Swiss mice.

Those pairs in which the males had received 20 or 30 percent D_2O and which had no litters during the first 28 days began having litters after 45 days of mating, indicating that the sterility produced by D_2O is slowly reversible (*8*).

We found no significant difference in the litter size or the sex ratio from that of the controls. This is in contrast to the effect of radiation which shows, in addition to the sterility effects (*9*), reduction in the litter size and a change in sex ratio (*10*). The failure of Hansen and Wülfert (*11*) to observe sterility in mice as a result of administration of D_2O is probably due to the low concentration employed by them.

Table 1. Effect of D_2O on the fertility of C_{57} and Swiss mice. D_2O was added to the drinking water.

Sex	D_2O concn. (%)	Pairs (No.)	Pairs Sterile (%)	Offspring Av. No.*	Offspring Av. No. per mating
		C_{57} mice			
Controls	0	24	17	6.4	4.4
Male	5	10	30	5.4	3.8
Female	5	10	30	7.6	3.8
Male	30	19	100	0	0
Female	30	10	40	5.2	2.6
		Swiss Mice			
Controls	0	19	5	9.0	8.5
Male	20	10	40	9.3	5.6
Female	20	11	0	9.1	9.1
Male	30	10	100	0	0
Female	30	10	0	9.1	7.3

* Calculated as the total number of offspring surviving 2 weeks divided by the number of litters containing live offspring at 2 weeks.

Several physiological mechanisms by which D_2O produces sterility in mice can be suggested. D_2O could interfere with maturation of the ova or sperm, or possibly reduce sperm motility. There is also an indication from our results that D_2O may interfere with the proper development of the fertilized ovum. It has been shown that D_2O inhibits the cell division of algae (*2*). This observation suggests that the most likely points of susceptibility would be the development of the sperm and the division of the fertilized ovum. That the former may be the more probable is supported by the greater susceptibility of the males to D_2O and, theoretically, by the known difference between males and females in the generation of germ cells.

On the biochemical level, hydrogen bonding is important for the maintenance of the secondary and tertiary structure of many biologically important macromolecules. Even a small difference in the properties of a proton bond and a deuteron bond might be expected to induce

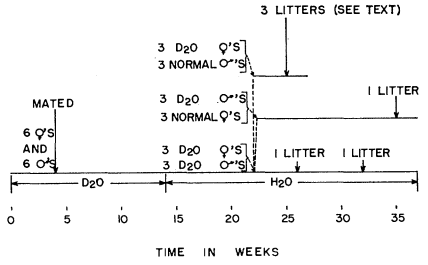

Fig. 1. Production of sterility in C_{57} mice by administration of 30 percent D_2O in the drinking water. Horizontal arrows indicate the length of time animals were given D_2O or H_2O. Vertical arrows indicate the time of mating or littering. Dotted lines indicate the time of cross-mating of treated and normal animals, as described in the text.

large effects in macromolecules and particularly in their function. Aberrations in the structure of deoxynucleic acids may be of especial importance because of the role of these acids in gene structure and cell division.

Further work is in progress to find other physiological effects of D_2O and to understand the mechanism of these effects in biochemical terms.

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4. The work described in this report was sponsored by the U.S. Atomic Energy Commission.
5. All D_2O dilutions were made up as volume-percent, approximately equal to atoms-percent of the total hydrogen present.
6. As used in this paper, the term *mated* indicates that males and females were housed together continuously.
7. *Sterility,* as used in this paper, means the inability to produce visible pregnancy.
8. *Note added in proof.* These mice sired five litters which were born 45 to 49 days after D_2O administration was discontinued, 18 litters which were born during the 57- to 76-day period following withdrawal of D_2O, and only three litters which were born in the following 5-week period. Females of the five remaining pairs were not pregnant at 105 days [W. L. Russell, "Genetic effects of radiation in mammals," in *Radiation Biology,* A. Hollaender, Ed. (McGraw-Hill, New York, 1954), vol. 1, pt. 2, pp. 825–859].
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Dating of Zawi Chemi, an Early Village Site at Shanidar, Northern Iraq

Briefly mentioned by Robert Braidwood in his article, "Near Eastern Prehistory" (*1*), the open village site of Zawi Chemi Shanidar, situated in northern Iraq, has recently provided material for a carbon-14 date. The charcoal sample was dated as $10,870 \pm 300$ years before the present at the radiocarbon laboratory of the U.S. Geological Survey and bears the laboratory number W-681. This site, tested under Smithsonian Institution sponsorship (*2*), has two occupations, totaling a depth of 1.5 m. The upper, layer A, is of post-Christian date. The lower, layer B, in which the sample was recovered, contained a preceramic industry which may be generally equated with "early Neolithic" or Braidwood's "incipient cultivation." The sample (*3*) was picked from a broad charcoal streak at a depth of 1.2 m, well within layer B. Some tree rootlets were observed in various parts of the excavation, but no contaminating rootlets were seen in the immediate area of the sample.

A few centimeters beneath the locus of the sample and to one side was found a roughly circular enclosure about 3 m in diameter, composed of river cobbles and field stones. It looks like an example of primitive architecture. The carbon date would appear to give an approximate age for this feature.

The same material culture was found in the upper stratigraphy of Shanidar Cave, about 4 k away, which provides a reasonable basis for assuming contemporary or seasonal occupations at both sites. Furthermore, an age of $10,600 \pm 300$ years before the present (W-667) was determined from a charcoal sample from the top of layer B of the cave, or Shanidar B1. This is just beneath and somewhat intermixed with material from the base of layer A, where close resemblances with Zawi Chemi B are found.

Charcoal is known to absorb carbon from humic acids in circulating ground water. This contaminating carbon can be older or younger than the charcoal, depending on its source, but a younger source is more likely in most situations. For this reason, samples being prepared for radiocarbon dating are boiled in solutions of HCl, then in NaOH, and finally in HCl again. The material extracted in the alkali treatment consists of the humic acid and lignin fraction which can contain the transported "foreign" carbon. This portion is not included in the C^{14} analysis. Generally, only the remaining material is used. However, the sample from the open village site (W-681) was found after separation to be too small for an analysis, and so both fractions were combined for the run. The error quoted after the age does not include the possibility of foreign contaminants, which is impossible to assess, but as is customary, merely gives the counting error due to random disintegrations. The sample from the cave (W-667) yielded sufficient material for a normal analysis.

Karim Shahir, an open site excavated by the University of Chicago Oriental Institute team and situated about 160 k to the southeast, has an industry which is rather like that from Shanidar Cave and the Zawi Chemi village site. We can say tentatively, on the basis of the present evidence, that Karim Shahir, the related sites, and the Shanidar occupations are culturally as well as chronologically related (*4*).

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4. Publication authorized by the director, U.S Geological Survey, and by permission of the secretary, Smithsonian Institution.

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Nucleolar Chromosome in the Rust Fungus Scopella gentilis

Allen (*1*), while working with *Puccinia malvacearum* Bert., first surmised that in rust fungi the nucleolus is probably organized on some definite locus of a chromosome. Olive (*2*), in a cytological study of *Coleosporium vernoniae* Berk. & Curt., noted that during the early stages of meiosis a nucleolus organizing chromosome is discernible. The morphological details of the chromosome as found in rust fungi, however, do not seem to have been fully elucidated. Singleton (*3*), in *Neurospora crassa* Shear & Dodge, has demonstrated the occurrence of a satellite chromosome associated with the nucleolus during mitosis as well as meiosis. The satellite zone [SAT-zone (*4*)] during pachytene, however, was not clearly demarcated, and it was observed that either due to the close proximity of the "b chromomere" to the satellite or due to its being distantly located, the latter frequently lost its identity. In the chromosome map of *N. crassa* this was designated "chromosome 2" because it was the second longest in a complement of seven.

In the study reported here (*5*) it was determined that *Scopella gentilis* (Syd.) Mundk. & Thirum. possesses a haploid complement of eight chromosomes. It was found that in early diplotene the nucleolar chromosome pair, because of its distinctive morphology, could be easily differentiated from the other seven pairs (Figs. 1–3). Inasmuch as the present observations are based on aceto-orcein preparations, the nucleolus is barely visible as an unstained globular refractive body (Fig. 3, arrow). The satellite-zone consists of a pair of stalked satellites or trabants followed by a swollen knoblike region. Its resemblance to the heterochromatic region found in the nucleolar chromosomes of certain higher plants is quite suggestive. The short arm, apart from bearing the nucleolus organizer, also possesses two interstitial chiasmata (Fig. 2). A small achromatic gap can be observed just where the long arm of the chromosome begins (Fig. 2, arrow) which possibly denotes the centromere position. The chromosome in diplotene measures about 8.5 μ, and it is the longest pair in the whole complement. In the upper focus of the microscope the