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

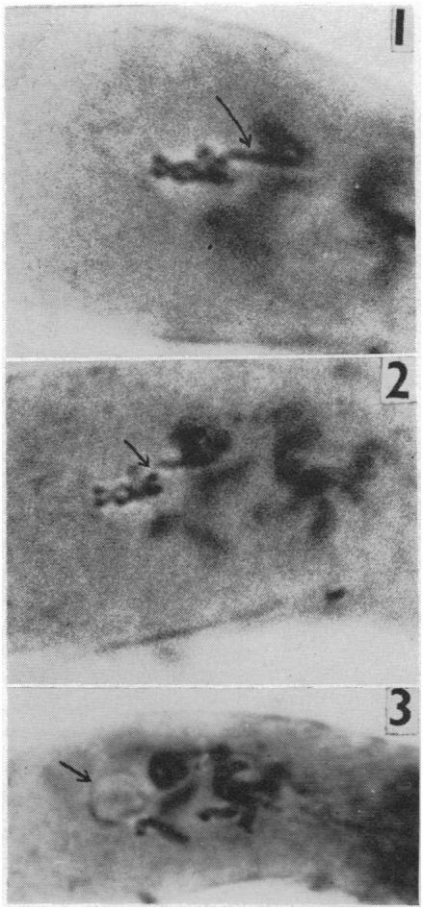

Fig. 1. Part of a basidium showing nucleolar chromosome in diplotene with its long arm (arrow) in full focus and its satellite zone partly in focus. (About × 4040.) Fig. 2. Same as Fig. 1, with short arm in full focus, showing satellite zone and interstitial chiasmata; arrow indicates possible location of centromere. (About × 3760.) Fig. 3. Same as Figs. 1 and 2, showing relative position of the nucleolus (arrow). (About × 1880)

nucleolus has been observed to cover the short arm of the chromosome in its entirety (Fig. 3). It seems likely that the nucleolus is organized by the so-called nucleolar constriction region of the chromosome. Matsuura (*6*) has distinguished two types of nucleolar chromosomes in the higher plants: the interstitial type and the terminal type. The nucleolar chromosome observed in *S. gentilis,* if this distinction is followed, belongs to the interstitial type.

As is well known, the morphological features of this chromosome are better revealed in the plant cells. The fungal nuclei have been considered by some investigators, such as Olive (*7*), to be more or less similar to those of the higher plants. The finding of a nucleolar chromosome with distinctive morphology of its own would appear to substantiate this view.

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4. The abbreviation "SAT" signifies, in addition to "satellite," "*sine acido thymonucleinico*" (without thymonucleic acid); since it has both connotations, the abbreviated form "SAT-zone" is widely used in current cytological literature.
5. I am indebted to the National Institute of Sciences of India for awarding me a research fellowship during the tenure of which this work was performed. Grateful acknowledgments are also due to Dr. S. P. Agharkar for laboratory facilities and encouragement, to Drs. M. J. Thirumalachar and G. B. Deodikar for helpful suggestions, and to Dr. S. H. Tulpule for furnishing advice and literature on cytology.
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Histochemical Localization of Acid Phosphatase in Bone Tissue

Analysis of the histochemical distribution of an enzyme is a valuable method for understanding and interpreting its physiological role. There is an extensive series of papers on the histochemical distribution of alkaline phosphatase in bone tissue, especially on its distribution during bone formation (*1*), and on the basis of these data and others of a biochemical character, some hypotheses have been put forward with respect to the action of alkaline phosphatase—namely, that its action takes place preponderantly in the cartilaginous calcification and formation of bone matrix and its later calcification.

Biochemical data exist relative to the presence of acid phosphatase in bone tissue; the increase of acid phosphatase in metastasic zones of some carcinomas (carcinoma of the prostate, for example) accompanied by a rise in the level of this enzyme in serum is known (*2*).

Although the histochemical localization of acid phosphatase in diverse tissues and organs has been investigated, because of technical difficulties, its localization in calcified tissues has not been studied in detail.

Recently we made a thorough analysis to establish the basic conditions for making a correct decalcification of bone tissue in order to show the above-mentioned enzyme. On the other hand we proved that the technique for demonstrating this enzyme could not be applied if even a vestige of calcium remained in the slides; chelating agents were tried but with no success.

Following these studies, we started a systematic study of the histochemical distribution of this enzyme in the normal ossification processes in man, rats (stock), and mice (strains C_3H and BAL). Numerous pathological specimens showing osteogenic phenomena and destruction of bone tissue were also used. All the material was treated, after being fixed in neutral 10-percent formalin for 24 hours at 4°C, in a buffer solution of 5-percent formic acid and 20-percent sodium citrate in equal parts, during a preliminary period, until all calcium was eliminated. Later on a modification of Gomori's technique (*3*) and the azo-dye method recently developed by Burton (*4*) were used on the frozen sections. The two techniques gave comparable results insofar as topographic and histological localization was concerned, and the differences in details of secondary importance, of a cytological order, were minimal.

Acid phosphatase was shown to be present in large quantities in the giant cells found in the proximity of erosive bone surfaces (osteoclasts) and cartilaginous surfaces (chondroclasts); we also found large quantities of enzyme in the walls of the vessels adjacent to erosive surfaces. The behavior of the enzyme was similar in the three species studied. In the pathological cases, an association of this enzyme with areas of bone reabsorption was evident, the enzyme being also found in abundance in the multinuclear giant cells of giant cell tumors and other related processes—cells which show a relationship to osteoclasts, even though this be only morphologic (Fig. 1).

In conclusion, we can report that, by using an adequate technique for decalcification, it is possible to show, easily and in a consistent and regular fashion, acid phosphatase in the hard tissues,

Fig. 1. Enchondral ossification zone in the limb of a newborn infant [Gomori's acid phosphatase techniques (frozen section); incubation time, 10 minutes]: (*a*) proliferating cartilage; (*b*) hypertrophic and calcified cartilage; (*c*) bone trabeculae in formation. The most intense enzymatic activity is observed in chondroclastic (*d*) and osteoclastic (*e*) cells. The hypertrophic and calcified zone is lacking in enzyme. (× about 120)