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55. Various materials are used for bonding the crystal transducer to the sample; these include glycerine, Nonaq, Dow Corning 200 silicone oil, epoxy resins, indium, and General Electric 7031 adhesive. The sample is maintained at low temperatures (see Fig. 6).
56. Support of microwave ultrasonic research in this laboratory by the National Science Foundation and the Office of Naval Research is gratefully acknowledged.

Deuterated Organisms: Cultivation and Uses

Living organisms of unusual isotopic composition can be used for magnetic resonance studies.

Joseph J. Katz and Henry L. Crespi

The element hydrogen occurs in nature as a mixture of nuclei, identical in charge but differing in mass. The predominant light variety of hydrogen, of mass 1, is accompanied by a rare, nonradioactive, heavy isotope of mass 2, which occurs in the proportion of 0.015 parts per 100 parts of ordinary hydrogen. Although, in most cases, the isotopes of an element are very similar in chemical properties, the hydrogen isotopes differ between themselves to an extent that justifies individual names, and the heavy, nonradioactive hydrogen isotope of mass 2 is called deuterium. Hydrogen is present in water and all organic compounds, and is thus an essential component of all living matter. By a "deuterated organism" we mean one in which all the hydrogen present is in the form of the heavy isotope. Not only the cellular water but the cellular components contain deuterium instead of hydrogen. A deuterated organism thus has an unusual relationship to its prototype organism. A deuterated organism is an artifact, for it is to be found nowhere in nature. The hydrogen-containing and the deuterated organisms may differ significantly in morphology, cytochemistry, and biosynthetic capacity, but basically they must be the same organism. By our definition, a deuterated organism is essentially free of ordinary hydrogen, and is able to carry out all metabolic activities essential to life. It is the purpose of this article to describe how deuterated organisms may be grown and how they differ from their prototypes, and to indicate some of the uses to which such organisms (and the compounds that can be derived from them) may be put.

Deuterium was discovered by Urey, Brickwedde, and Murphy (*1*) in 1932. That deuterium would have special significance in biological systems was recognized very shortly after its discovery. The hydrogen isotopes differ more in chemical properties than the isotopes of any other element do (*2*). Consequently, the replacement of hydrogen by deuterium in water and in chemical compounds may result in considerable differences in reaction rate (*3*) and in changes in ionic equilibria (*4*), in water structure (*5*), and in various physical parameters such as vapor pressure (*6*) and infrared spectra (*7*). In more biological systems, substitution of deuterium can affect protein conformation (*8*) and coil-helix transitions (*9*). Some

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deuterium isotope effects in vitro have recently been reviewed by Bigeleisen (*10*), and deuterium isotope effects of biological importance have been reviewed by Thomson (*11*) and by our associates and ourselves (*12, 13*). Although deuterium isotope effects in vitro command attention, it is in the biological realm that these isotope effects are perhaps most impressive, because perturbations in rate or equilibrium which may be trivial in laboratory situations can assume a decisive character in intricate, highly organized, and delicately poised living organisms.

The very early experiments of G. N. Lewis (*14*) in 1933 and many subsequent studies made as late as 1960 all led to the conclusion that substantial replacement of hydrogen by deuterium was incompatible with life. This conclusion is still valid for higher plants and animals, but it is by no means valid for all living organisms. Indeed, so numerous are the known exceptions that there is now a legitimate basis for the hope that there may be no ultimately insurmountable barrier to the cultivation of higher plants and animals in deuterated form. In 1960, Chorney *et al.* (*15, 16*) were successful in growing the green algae *Chlorella vulgaris* and *Scenedesmus obliquus* in media containing 99.7 percent D_2O with carbon dioxide as the sole carbon source, and since then many other algae, bacteria, molds, and yeasts, and even a motile protozoon-like organism, have been adapted to growth as fully deuterated organisms. The current status of these studies is shown in Fig. 1.

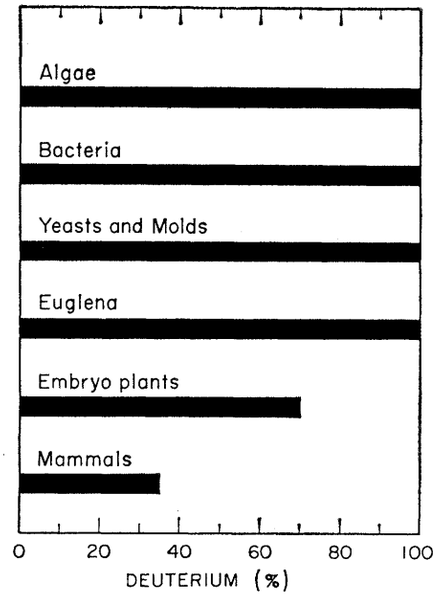

Fig. 1. The extent to which hydrogen can be replaced by deuterium in representative members of various classes of organisms.

Deuterated Algae

Unicellular green algae were the first organisms to be grown in fully deuterated form. Many algae, being photosynthetic organisms, are capable of full autotrophic growth in a purely inorganic environment. Provision of nutrient media free of ordinary hydrogen is thus relatively simple, for water is the only hydrogen-containing compound required, and heavy water (D_2O) is available on an industrial scale (*17*). A nutrient solution made of D_2O, inorganic salts (nitrate or other nitrogen source, phosphate, sulfate, Na^+, Ca^{++}, K^+, Mg^{++}, and trace elements), and carbon dioxide is sufficient to satisfy the nutritional requirements of many autotrophic algae. Heavy water, at least when the algae are first exposed to it, generally has a strongly adverse effect on cell reproduction (*18*) and growth, if the D_2O is present in the medium at a concentration higher than 75 percent, and as the concentration of D_2O is further increased, multiplication virtually ceases. Nevertheless, for *Chlorella vulgaris* and *Scenedesmus obliquus*, after adaptation periods that range from 10 days to a month or more, growth and reproduction begin in D_2O, and on subsequent transfer of the algae to 99.7 percent D_2O, growth begins without lag. Cultures may then be maintained in D_2O for periods of years.

It has proved possible to grow the following green algae autotrophically in media containing 99.7 percent D_2O: *Chlorella vulgaris, C. ellipsoidea, C. pyrenoidosa, C. pyrenoidosa* (high-temperature strain), and *Scenedesmus obliquus*. Blue-green algae have also been grown successfully: *Fremyella diplosiphon, Nostoc commune, Phormidium luridum, Plectonema calothricoides, Cyanidium caldarum*, and the thermophile *Synechococcus lividus*. The diatoms *Navicula pelliculosa* and *Phaeodactylum tricornatum* and the red alga *Porphyridium cruentum* have not yet been cultured successfully at D_2O concentrations greater than 75 percent. It appears altogether probable that many additional species of algae can be adapted to growth at high concentrations of D_2O should this become desirable.

Deuterated algae can be mass-cultivated on a continuous basis (*19*). Suitable equipment is indicated in Fig. 2. The algal cultures (unialgal, but generally not sterile) are contained in closed lucite (polymerized methyl methacrylate) boxes of 2.5- or 5-liter capacity. Temperature control is effected by heat-exchange channels milled into the bottom of the boxes, through which water at the appropriate temperature can be circulated. Agitation, which is essential for dense cultures, is achieved by rocking the boxes. The necessary light is supplied by fluorescent lamps, and each box is fed with a mixture of CO_2 (5 percent) and N_2 (95 percent). The growth rate is followed by measurement of packed-cell volume. At a high cell density but before the population becomes stationary, four-fifths of the culture is harvested and the box is then replenished with fresh nutrient. Cultures have been maintained in this way for as long as 3 years.

The growth rate of algae in D_2O is considerably lower than the rate for algae in H_2O. Under conditions of light saturation, a surprisingly constant deuterium isotope effect on the growth rate is observed, the normal rates being reduced by factors of about 3.3 to 3.9. To a first approximation, the magnitude of the deuterium isotope effect on growth appears to be independent of algal species, or even of temperature. There is no simple relation, further, between ease of adaptation and subsequent growth. As the cultures become more dense, light becomes rate-limiting, the growth rates decrease, and differences in growth rate between different algae and between algae grown in H_2O and D_2O diminish. In our mass-culture apparatus, *Scenedesmus* and *Chlorella* are produced at the rate of 1 to 2 grams (dry weight) per day per 5-liter box. Blue-green algae generally grow more slowly, but even with the slower-growing organisms, adequate growth rates can be achieved. Thus, the thermophilic blue-green alga *S. lividus* grows in D_2O at 50°C at a rate quite comparable to that of *Chlorella* growing in H_2O at room temperature.

Adaptation to growth in D_2O is a complicated affair. Many bizarre events are observed when algae are transferred from H_2O to D_2O. Most notable is the appearance of greatly enlarged or "monster" cells, which have been described by Calvin (*20*). In *Chlorella vulgaris*, cells with diameters up to 30 microns may be observed, and cells of

15-micron diameter are common; the size is striking, for ordinary cells of *C. vulgaris* are about 5 microns in diameter. In *Scenedesmus obliquus*, some cells attain diameters two or three times normal. Monster cells contain extraordinarily large amounts of nucleic acids, as revealed by cytochemical studies (*21*). The increase in cell size in *C. vulgaris* is accompanied by a proportionately greater increase in the size of the nucleus and chloroplasts, and, as a result, a much greater fraction of the total volume of the cell is occupied by these structures. The nucleus and chloroplasts change in size and shape, becoming multilobate and inconsistent from cell to cell. The appearance of monster cells in adapting cultures may be regarded as evidence of serious difficulties posed by the deuterium environment. Monster cells are evidence of failure to adapt, and are not on the main route to cultivation of deuterated organisms.

Some preliminary observations (*22*) suggest that adaptation of hydrogen-grown algae to growth in D_2O may be considerably facilitated by the addition of supernatant solution from D_2O cultures. We have purified and fairly well characterized a substance from spent D_2O culture media which appears to facilitate considerably the initial adaptation of hydrogen-grown algae to D_2O. The substance is nondialyzable and very heat-sensitive. It appears to have the properties of a glycoprotein, and when studied by electrophoresis and sedimentation analysis, seems to be homogeneous. Introduction of a few micrograms into a fresh D_2O medium has a remarkable, accelerating effect on adaptation of hydrogen-grown *Chlorella vulgaris*, *Scenedesmus obliquus*, and *Phormidium luridum* to growth in D_2O. This substance seems to be absent in the supernatant solution of H_2O cultures. The fact that this "adaptation factor" is of high molecular weight and is not, as far as we can determine, species-specific only complicates the picture of the adaptation process further.

Is adaptation to growth in D_2O a somatic adaptation or a genetic mutation? The evidence in favor of somatic adaptation seems to us quite conclusive. Subculture of *Chlorella* or *Scenedesmus* from H_2O to D_2O tends strongly to induce synchronous growth. Under identical conditions, adaptation times are reproducible for a particular species. The lag period, on subculture to D_2O, is observed to vary directly with

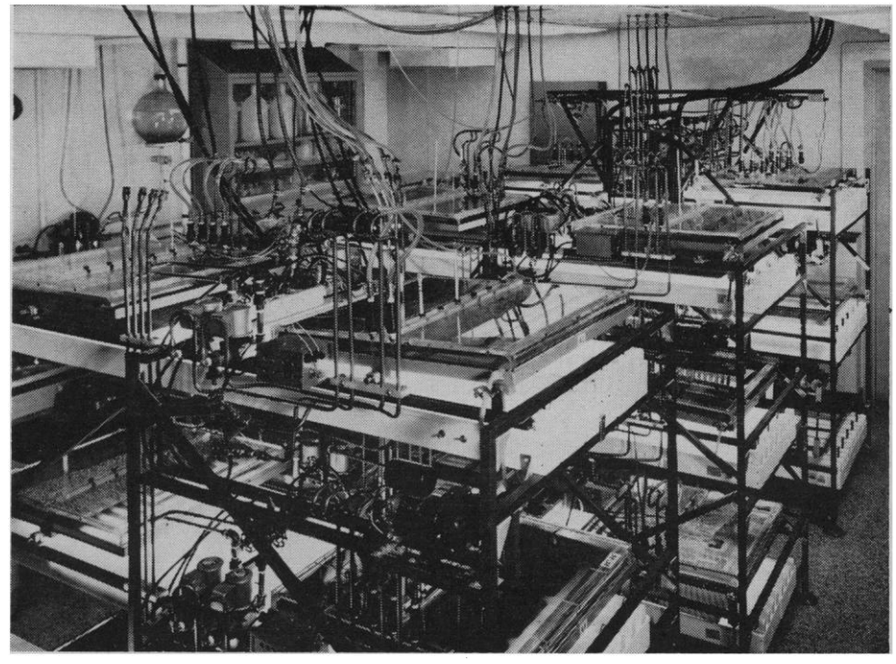

Fig. 2. View of a farm for growing deuterated algae, showing rocking boxes, lights, and auxiliary equipment. [Designed and assembled by Homer F. DaBoll]

D_2O concentration. Finally, transfer back to H_2O from D_2O results eventually in an organism of normal growth characteristics and morphology. Such a de-adapted organism readapts to D_2O no more rapidly than organisms that have never been exposed to deuterium.

Orgel (*23*) has considered the general problem of adaptation to widespread disturbance of enzyme function, which presumably is an important factor in adaptation to deuterium. Isotope effects must be associated with a large fraction of the chemical events that take place in the cell, and it is hard to see how the number of mutations which would be required if adaptation were a genetic process could occur in the relatively short adaptation period. Therefore, adaptation, in general, must be a somatic process and must include a relaxation of repressor control of enzyme synthesis. The ability to grow in D_2O must be implicit in many organisms. Those organisms that have sufficiently responsive control systems can successfully survive adaptation. This generalization, however, has practical limitations because of the extreme complexity of enzyme inducer-repressor systems, and it is therefore not surprising that the cultivation of organisms in D_2O is still to a very considerable extent an empirical art.

De-adaptation of deuterated organisms back to H_2O is also a complex matter and does not necessarily parallel the original adaptation to D_2O. *Scenedesmus obliquus*, which adapts to D_2O with difficulty, can be restored to H_2O without adverse effects, whereas *Chlorella* species which adapt to D_2O readily, suffer severe disturbances in photosynthesis and respiration on transfer from D_2O back to H_2O.

Deuterated Bacteria and Fungi

Photosynthetic organisms are the only ones that can utilize carbon dioxide as their sole carbon source. All other heterotrophic organisms must be supplied with carbon-hydrogen compounds for growth. Before methods for mass-cultivation of deuterated algae were developed, provision of a fully deuterated culture medium for heterotrophic organisms was a practical impossibility. Because deuterated algae produce carbohydrates, amino acids, and other essential nutritional factors, complex culture media free of hydrogen are now readily available, and nutritionally fastidious and demanding organisms can be cultured in fully deuterated form.

It turns out that, in many cases, it is not necessary to isolate pure deuteriocarbohydrates, deuterio-amino acids, and the like to produce adequate media for culture of deuterated organisms. Various extracts can be prepared from deuterated algae, and these extracts

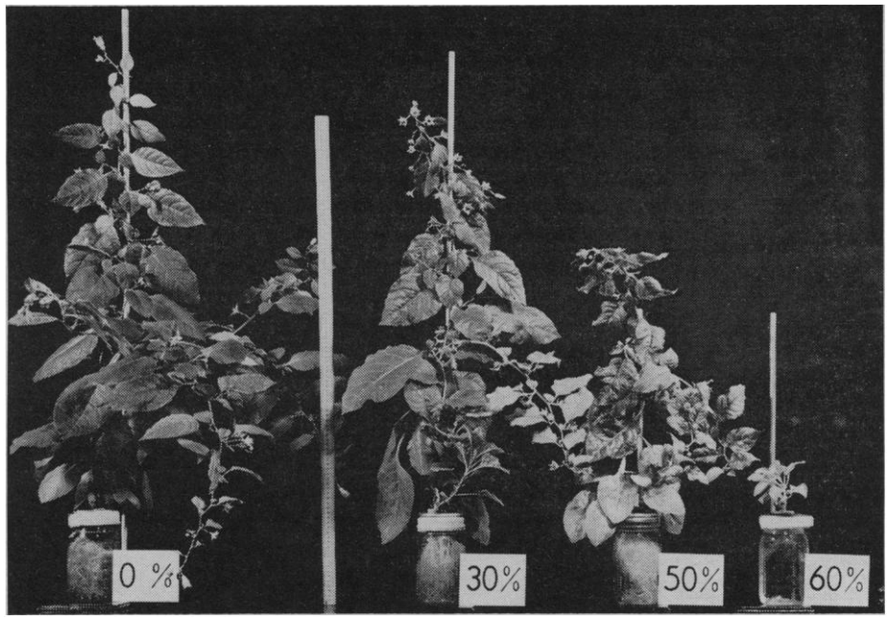

Fig. 3. Plants of *Atropa belladonna* grown hydroponically in nutrient solutions containing increasing concentrations of D_2O. [Uphaus *et al.* (*29*)]

serve as suitable nutritional supplements in D_2O (*24*). Use of such extracts has been found to simplify the cultivation of deuterated heterotrophs, and these substances are particularly useful in the culture of organisms for which fully defined culture media cannot yet be specified.

Many kinds of bacteria have now been grown in fully deuterated form: *Escherichia coli* (strains K12; B; C-600, permease$^-$ thr$^-$ leu$^-$ thiamine$^-$; and 3300 thiamine$^-$); *Bacillus tiberius; B. subtilis; B. cereus; Hemophilus influenzae; Serratia marcescens*; and *Rhodospirillum rubrum*. Johnstone (*25*) has successfully grown *Azobacter agilis* and *A. vinelandii* in D_2O with deuterio-glucose and deuterio-acetate as substrates.

Adaptation of bacteria to a deuterium-containing medium and their growth in that medium have some unusual features. In the case of *Escherichia coli* K12 growing at 31°C, replacement of H_2O by D_2O considerably increases the lag time and the doubling time, whereas substitution of deuterio-glucose for ordinary glucose in either H_2O or D_2O has only a minor effect. At 37°C, however, the situation is quite different, for deuterio-glucose has a very much more pronounced adverse effect on growth in D_2O than has either ordinary glucose in D_2O or deuterio-glucose in H_2O. In general, microorganisms appear to have considerably more difficulty in adapting to D_2O than in adapting to deuterio- substrates. If the organism can be induced to grow in D_2O with hydrogen substrates, it is probable that replacement of the hydrogen substrate can be effected. Deuterated bacteria have proved to be useful sources of fully deuterated DNA (*24*).

The fungi are an extremely large and extraordinarily varied group of organisms, distinguished by their biosynthetic prowess. The case of the yeast *Torulopsis utilis* illustrates the problems encountered in growing this class of deuterated organism. Normally, *T. utilis* grows in an H_2O medium composed only of glucose and inorganic salts; unlike the case for many other yeasts, no dietary factors are required. In D_2O, *T. utilis* becomes more demanding: no growth is observed in a D_2O medium containing deuterio-glucose and the usual salts. It has been shown that the only addition that need be made to the D_2O medium for good growth to occur is a very small amount of thiamine (*26*). Closer study has revealed that it is only the thiazole moiety of the thiamine molecule that is required. In D_2O, *T. utilis* loses its ability to synthesize the thiazole portion of thiamine but not the pyrimidine portion, and the organism retains the ability to combine the two portions of the thiamine molecule. The thiamine requirement for growth in D_2O persists even after protracted periods of such growth; on its return to H_2O, the yeast resumes the synthesis of thiamine, and an exogenous supply is no longer required.

Enhancement of nutritional fastidiousness occurs also with other yeasts. The brewers yeast *Saccharomyces cerevisiae*, which requires added thiamine and biotin for growth in H_2O, develops a further requirement for pantothenic acid and inositol for growth in D_2O. Extracts from deuterated algae provide an adequate source of these and other deuterated dietary factors.

The mold *Aspergillus niger* can be grown in D_2O on a mixture of deuterio-glucose and deuterio-algal extracts containing principally a mixture of deuterio-amino acids. Various strains of *Penicillium* have also been successfully grown in deuterated form, and, as in the case of bacteria, there is good reason to suppose that many more molds can be cultured in fully deuterated form. An example of one of these is the alkaloid-producing mold *Claviceps purpurea* (ergot) (*27*). Full deuteration of this organism has been achieved in a D_2O medium containing deuterio-monosaccharides, deuterio-succinic acid, traces of vitamins, and phosphate in low concentration. The organism grows well and forms an off-white surface pellicle, but its ability to produce alkaloids appears seriously impaired.

Higher Organisms

Higher plants and even the simplest animals resist full deuteration. Therefore the finding that the unicellular organism *Euglena gracilis* (strain Z) can be adapted to growth in 99 percent D_2O on fully deuterated nutrients (*28*) is of particular interest. Serial subculture over a period of months into progressively higher concentrations of D_2O is required. The organism appears to be more sensitive to the amount of deuterium in the water than to the isotopic composition of the substrate sugar. Deuterated *Euglena* organisms are shorter and broader than normal organisms. They appear more granular and have fewer and smaller chloroplasts. The flagella and motility appear unaffected. The eyespots of the young deuterated cultures are very faint, but pigmentation increases as the cultures age. The usual phototactic response is exhibited. *Euglena* is the first organism with distinct animal characteristics in which more than 99 percent of the hydrogen has been replaced by deuterium. This may be an augury for the future, but to judge from the prolonged adaptation period required in *Euglena*, adaptation of more complex organisms may prove to be no small challenge.

Neither higher plants nor animals survive anything like full replacement of hydrogen by deuterium. Since this discussion is focused on fully deuterated organisms, the work of the past few years on deuterium effects in higher plants and animals is mentioned here only briefly (*29*). As far as we know, no higher plant has been induced to grow well in D_2O concentrations much above 50 percent. Figure 3 shows the effects on the growth of belladonna of increasing the deuterium concentration. Strong inhibition is obvious above a D_2O concentration of 60 percent. At concentrations up to 50 percent, flowering and berry formation occurred, but in the more highly deuterated plants the berries were smaller and contained fewer seeds. At a D_2O concentration of 60 percent, no flowering occurred.

The most intensive effort yet made to adapt a higher plant to growth in D_2O has been made with duckweed (*Lemna perpusilla*) (*30*). This common aquatic plant can be grown heterotrophically in defined liquid media, so investigations with deuterium are readily performed. Abnormalities seen in plants grown in medium containing D_2O at concentrations of 50 to 63 percent are to a considerable extent eliminated by the addition of kinetin. There is an extraordinary difference in the response to D_2O concentrations of 63 and 65 percent; growth occurs at a concentration of 63 percent, but at 65 percent growth is completely inhibited.

Numerous experiments have been carried out on the deuteration of higher animals, mainly in connection with the possible utilization of deuterium isotope effects for the control of tumor growth (*31*). When more than about one-third of the hydrogen in the body is replaced by deuterium, the animal, be it mouse, rat, or dog, suffers severe and usually fatal physiological disturbances (*32*). The physiology and pathology of deuterium substitution in mammals are exceedingly complex and not well understood (see *11* for details).

Morphological Consequences of Deuterium Substitution

Major changes in the isotopic composition of living organisms may result in gross changes in the structure of cells and organelles. Electron microscopy shows the chloroplasts from deuterated algae to be far less ordered than their hydrogen prototypes (*13*).

Table 1. Effect of deuterium substitution on the dominant signal seen in the light in a variety of microorganisms. [After Kohl *et al.* (*39*)]

Organism	Line width (ΔH_{ob}) (gauss)*		$\Delta H_{ob-H}/\Delta H_{ob-D}$†
	Deuterium	Hydrogen	
Rhodospirillum rubrum	4.5	8.8	1.95
Synechococcus lividus	3.6	7.3	2.03
Chlorella vulgaris	3.0	7.5	2.50
Scenedesmus obliquus	2.5	9.5	3.80
Chlamydomonas reinhardti	3.0	~9	3

* ΔH_{ob} is the distance (in gauss) from peak to peak of the derivative curve. † $\Delta H_{ob-H}/\Delta H_{ob-D}$ is the ratio of ΔH_{ob} for the spectrum generated by the hydrogen culture to ΔH_{ob} for the spectrum generated by the deuterium culture.

The morphological sequelae of deuterium substitution are also evident in Fig. 4, which shows both hydrogen-containing and deuterated bacteriophage T2 (*22*). The deuterated bacteriophage particles are larger and more fragile, and the electron photomicrographs strongly suggest that the protein subunits in the tails of the bacteriophage are disordered. Reduced morphological order seems characteristic of all deuterated microorganisms so far examined.

Deuterated Organisms in Magnetic Resonance Spectroscopy

Not only are deuterated organisms interesting in their own right but they can be put to a variety of research uses. Deuterated organisms serve as a practical source of deuterium compounds, many of which would be difficult or impossible to obtain by conventional synthetic procedures. Fully deuterated sugars, amino acids, chlorophylls, carotenoids, proteins, and the like have been prepared from appropriate deuterated organisms (*33*) and have been used for the investigation of unusual deuterium isotope effects. Thus, mutarotation has been studied with deuterio-glucose and deuterio-mannose (*34*); hydrophobic bonding has been studied with deuterio-proteins (*35*), and chlorophyll aggregation with deuterio-chlorophyll (*36*).

It is in the applications of magnetic resonance spectroscopy, however, that isotopic composition is of decisive importance. In electron spin resonance and nuclear magnetic resonance spectroscopy, the isotopes present in the substance under examination are a primary factor in the observed spectra. Isotopic replacement is, of course, widely used to facilitate the interpretation of these spectra. The compounds obtainable from deuterated organisms, however, make it much more practicable to bring this powerful technique to bear on compounds of biological importance. In this article, therefore, we emphasize ways in which deuterated organisms can be used in conjunction with proton magnetic resonance spectroscopy.

The magnetic properties of the hydrogen nucleus make it possible to classify the hydrogen atoms in an organic compound by number and kind. The magnetic properties of hydrogen and deuterium are very different, and, under conditions where protons can be observed, deuterons are invisible. The introduction of hydrogen by either chemical or biochemical reaction into deuterio- compounds can thus be readily detected by proton magnetic resonance spectroscopy. A means is thus available for observing the path of hydrogen in biological systems. Proton magnetic resonance reveals both the site of the hydrogen in the molecule and its relative amount; with this method, unlike carbon-14 or tritium tracer techniques, no extensive chemical degradation is required for determining where the hydrogen is located in the molecule into which it is incorporated.

Hydrogen can be introduced into the constituent compounds of a deuterated organism in various ways: (i) the autotrophic organism can be grown in a mixture of H_2O and D_2O; (ii) the organism can be grown in D_2O on carbon-hydrogen substrates; (iii) the organism can be grown in H_2O on carbon-deuterium substrates; (iv) the deuterated organism can be transferred from D_2O to H_2O. Each of these procedures relates to a somewhat different aspect of the metabolic activities of the organism. Algae grown in a mixture of H_2O and D_2O can be used to obtain information on kinetic isotope effects in biosynthesis. From the magnitude and sign of the isotope effect, conclusion can be drawn about biosynthetic pathways. Chlorophyll biogenesis in green algae has been studied in this way. Procedures ii and iii likewise yield information on biosynthetic pathways, and procedure iv has been used to follow the path of hydrogen in photosynthesis. We have carried out experiments of all these kinds, but only two examples are discussed here.

Applications to Biosynthesis and Photosynthesis

The photosynthetic purple bacterium *Rhodospirillum rubrum* can be successfully adapted to growth in fully deuterated form. These bacteria utilize a carbon-hydrogen compound such as succinic acid, ordinary or deuterated, as a hydrogen donor. In an experiment on the biogenesis of bacteriochlorophyll (*37*), the organism is grown in D_2O on ordinary succinic acid and in H_2O on deuterio-succinic acid. A set of such "mirror image" experiments should yield bacteriochlorophylls that have isotopic compositions complementary to each other for all biosynthetic reactions that do not involve uncatalyzed hydrogen exchange of intermediates with the medium.

In fact, the two bacteriochlorophylls obtained in this way showed substantial differences from the ideal "mirror image" isotopic composition, and these differences cannot be explained as entirely due to isotope effects on the *de novo* synthesis of bacteriochlorophyll in the deuterated organisms (Fig. 5). It appears likely at this time that the biosynthetic pathway to bacteriochlorophyll is dependent upon the isotopic composition of the growth medium; from this the important deduction can be made that more than one biosynthetic pathway for the synthesis of bacteriochlorophyll is available to the organism.

The often suggested possibility that cyclic hydrogen transport occurs between chlorophyll and water during photosynthesis has been investigated with deuterated organisms and proton magnetic resonance (*38*). Deuterated *Scenedesmus obliquus* organisms growing in 99.7 percent D_2O were harvested by centrifugation and immediately resuspended in H_2O medium. The organisms were supplied with carbon dioxide and allowed to continue photosynthesis for up to 16 hours. The algae were again harvested, and the chlorophyll was extracted and purified by conventional means and then examined by proton magnetic resonance. The spectra clearly show that the chlorophyll contained no hydrogen at the exchangeable δ-methine position, or at positions 7 and 8 in ring IV. This result strongly suggests that exchangeable or labile hydrogen in chlorophyll is not directly involved in photosynthesis. These studies are being extended to carbohydrates and amino acids, and the results should help clarify the path of hydrogen in photosynthesis.

Electron spin resonance signals that increase on illumination of photosynthetic systems have been studied for some time, but the molecular environment of the unpaired electrons that give rise to the signal has not been definitely ascertained. The availability of isotopically substituted microorganisms permits a new approach to this problem. Where the isotopes have different nuclear magnetic moments or spin, isotopic substitution will influence the magnitude (for cases of different magnetic moment) or the multiplicity (for cases of different spin) of the signal generated by the interaction of the electron and nuclear spins. The line width and hyperfine structure of the

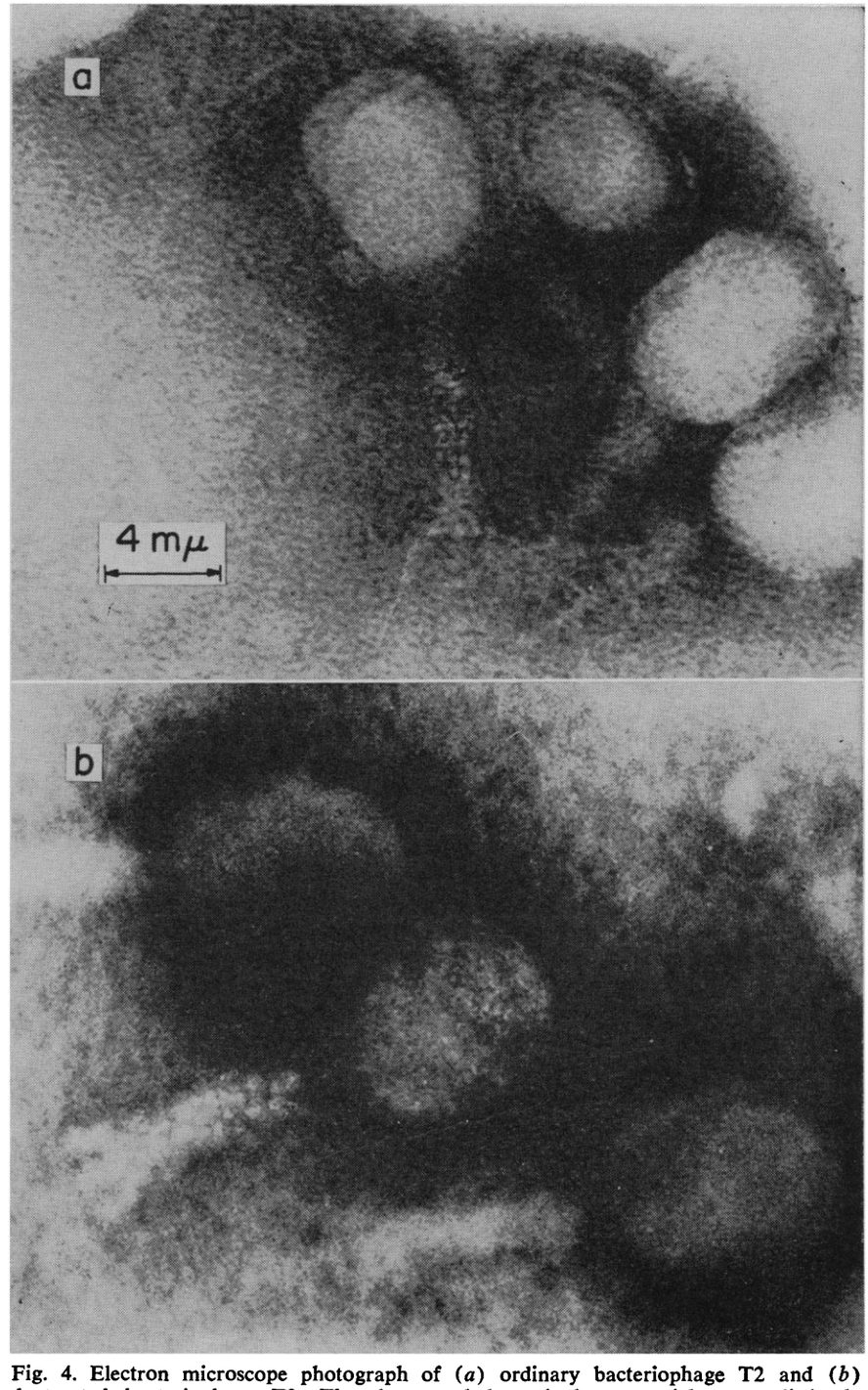

Fig. 4. Electron microscope photograph of (*a*) ordinary bacteriophage T2 and (*b*) deuterated bacteriophage T2. The deuterated bacteriophage particles are distinctly larger and appear to have more bulbous heads than their normal counterparts. [After Flaumenhaft (*22*)]

signal are thus changed by the introduction of deuterium (Table 1) (*39*). Electron spin resonance studies of isotopically permuted algae and photosynthetic bacteria give some basis for the view that the chlorophyll molecules do not necessarily provide the locus of the unpaired electron, as had been rather generally assumed.

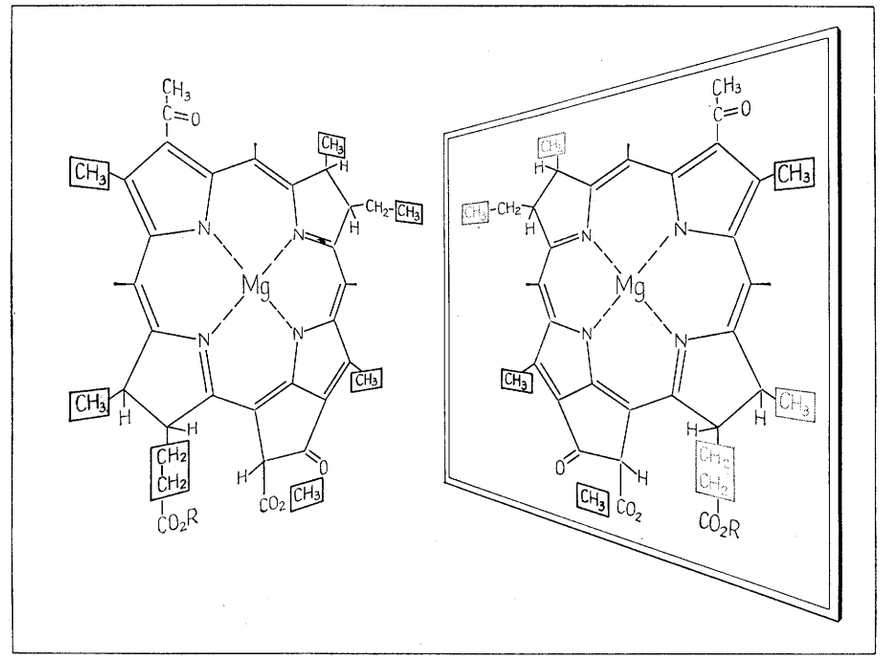

Fig. 5. An isotope "mirror image" experiment on the biosynthesis of bacteriochlorophyll. Bacteriochlorophyll is extracted from photosynthetic bacteria grown, in the one case, in H_2O on succinic acid-d_4 and, in the other, in D_2O on ordinary succinic acid. In the absence of exchange with the medium, the isotopic composition of the two chlorophylls should be mirror images. In fact, some groups have the expected composition but others do not. In the image (structure at right), three methyl groups have complementary isotopic compositions, but three other methyl groups and the $-CH_2-CH_2-$ group in the propionic acid side chain do not. This result implies a multiplicity of biosynthetic pathways to bacteriochlorophyll.

Deuterio-Proteins in Magnetic Resonance Spectroscopy

The problems of using proton magnetic resonance spectroscopy for studying proteins are well known (*40*). The anisotropy and slow motions of the large protein molecules make it difficult to average local magnetic fields, and only broad and ill-defined resonance bands are generally observed for proteins dissolved in water. Deuterio-protein, particularly phycocyanin, the photosynthetic protein pigment, can now be readily obtained from deuterated blue-green algae. Deuterio-phycocyanin has been studied in some detail, and considerable information has been accumulated about deuterium isotope effects on this protein (*35, 41*). It now appears that deuterio-proteins may provide some novel possibilities for protein studies by nuclear magnetic resonance techniques.

We have been able to observe the binding of ordinary (hydrogen-containing) benzylpenicillin by deuterio-phycocyanin. The hydrogen resonances in benzylpenicillin at 0.001*M* concentration in a 10 percent (by weight) solution of deuterio-phycocyanin in D_2O solution can be readily observed by on-line computer data collection. The resonances of the phenyl hydrogen atoms of the benzylpenicillin are observed to shift some 20 cycles per second (a shift of 0.2 part per million) to higher magnetic fields, relative to the resonances of the phenyl hydrogen atoms in benzylpenicillin in aqueous solution. This observation is in agreement with the conclusions of Fischer and Jardetzky (*42*) that the phenyl ring is the moiety involved in the binding.

We have also modified deuterio-phycocyanin through reaction with hydrogen-containing side-chain reagents (*43*) and have been able to detect the protons introduced into deuterio-phycocyanin by acylation with *p-tert*-butylbenzoyl chloride.

It appears to be possible to obtain deuterio-proteins with specified hydrogen amino acid side chains by biosynthesis. We may call such a protein an isotope hybrid. We have grown blue-green algae in D_2O in the presence of ordinary leucine, and find that the organisms can utilize exogeous leucine. Deuterio-phycocyanin extracted from organisms grown with exogenous leucine contains about one-tenth to one-fifth of its complement of leucine in the form of hydrogen leucine. (The incorporation of hydrogen leucine into deuterio-phycocyanin is established through examination of the hydrolyzate by proton magnetic resonance. Other hydrogen amino acids appear not to be present in significant amounts.) The leucine CH_3 groups in the hybrid deuterio-protein can be readily detected by proton magnetic resonance. Preliminary examination suggests that the resonances of the leucine methyl group are considerably shifted to higher field (relative to the resonances for simple amino acid dissolved in water). This can be taken to indicate that at least a portion of the leucine residues experience an environment in the protein radically different from the environment they experience in water. There are thus grounds for the belief that hydrogen-containing amino acid side chains incorporated into a deuterio-protein may function as reporter groups (*44*). These studies are consequently being extended to other amino acids.

Summary

Some of the manifestations of deuterium isotope effects in living organisms have been discussed. A considerable range of organisms can be grown successfully in fully deuterated form, and the prospects are good that many more organisms will be grown in this form.

Deuterated organisms are interesting in themselves, and they also are of importance to physical scientists. Fully deuterated compounds extractable from deuterated organisms can be used to study unusual deuterium isotope effects. Particular attention is given here to the use of deuterated organisms in conjunction with proton magnetic resonance spectroscopy for following the path of hydrogen in biological systems, for biogenetic studies, and for studies of proteins by nuclear magnetic resonance.

References and Notes

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The Accademia dei Lincei

Modern scientific societies owe many of their important traditions to the Lincean Academy, founded in 1603.

Stillman Drake

It would be hard to imagine modern science without scientific societies. The progress of scientific ideas is heavily dependent upon communication; hence the need for a particular kind of organization and a special class of publication. Scientific societies, by the selection of persons with highly specialized interests, greatly reduce the hazard that avenues of useful communication will be cluttered up with rubbish or damaged by false or misleading announcements in their fields. They also provide an important means for the organized defense of the interests of their members against interference with free research and communication and other disturbances from outside which occur from time to time.

Yet if it is hard to imagine modern science without scientific societies, from an a priori standpoint it would certainly seem that there must have been a time when science had to get along without those beneficial—I might say essential—organizations. Logically, there should have been a time when a few scattered scientists had begun the modern scientific revolution in thought, without any special society to act as a center of communication or to defend them from the onslaughts of their foes—and science has never existed without powerful enemies. One might reasonably expect the first scientific society to have been founded by scientists as they awakened to the need for mutual communication and mutual defense of their interests. And being reasonable people, you might therefore expect to hear from me the story of an early scientific society which came into being in that way. Instead, it is a story that seems (to me at least) rather improbable from an a priori standpoint—too improbable to be good fiction, as is the case with rather few events known to scientists, but with many known to historians.

Consider the probability that 6 or 7 years before the first startling discoveries and theories of modern science were published by Galileo and Kepler, and a dozen years before the first onslaught of established authority against

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