this would not account for the increased rate of protein synthesis observed after mitosis.

The system presented here offers potential for study of macromolecular events in mammalian cells having a high degree of synchrony during a particular stage of the cell cycle.

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6. In experiments on asynchronous populations it was found that pulse-labeling with C^{14}-amino acids for periods longer than 1 minute did not appreciably increase the C^{14} radioactivity in the polyribosome region of gradients but almost linearly increased the C^{14} radioactivity found near the top of the gradients. These results suggest that the 1-minute pulse label was sufficient to achieve steady state labeling of the growing polypeptide chains associated with polyribosomes.
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Remission of Aster Yellows Disease by Antibiotics

Abstract. *Suppression of symptoms of aster yellows by antibiotics supports the tentative hypothesis that the etiologic agent is a mycoplasma- or bedsonia-like organism rather than a virus. Development of symptoms was supressed by chlortetracycline, tetracycline, or chloramphenicol, but not by penicillin. When plants were treated with chlortetracycline at 1000 parts per million before symptoms appeared, symptoms developed only after cessation of the treatment. Assay of the agent of aster yellows, extracted from plants, indicated inhibition of growth of the pathogen by treatment with chlortetracycline. Plants severely affected before treatment began developed new symptomless axillary growth, including flowers; previously yellowed leaves often became green. Acquisition of the agent of aster yellows by leafhoppers was drastically reduced when infected plants were treated with chlortetracycline continuously for 1 week before exposure to the vectors. Our data, and preliminary evidence from purification studies, are consistent with a possible mycoplasma- or bedsonia-like etiology of the aster yellows disease.*

The causal agents of aster yellows, and similar plant diseases borne by leafhoppers, have long been considered viruses, a supposition based mainly on their filterability and their transmittability by grafting; yet they have long resisted isolation and visualization. Failures in purification have often been ascribed to instability in vitro, low concentration in infected tissues and cumbersome bioassay, but now it appears that the pathogen may not be a virus.

Japanese workers (*1*, *2*) proposed that yellows diseases of plants may be caused by mycoplasma- or psittacosis-like (bedsonia) organisms; their evidence was based on electron microscopy of diseased tissues and a therapeutic effect of tetracycline antibiotics. Early results (*3*) on sedimentation and filterability of the aster yellows agent, later work (*4*), and our own data from purification studies could be explained by a mycoplasma-like etiology of aster yellows.

We have examined this hypothesis with aster yellows. Here we summarize data from chemotherapy experiments (*5*) based on the tenet that aster yellows, if caused by a mycoplasma or bedsonia organism, should be amenable to treatment with certain "broad-spectrum" antibiotics.

A celery-infecting strain of aster yellows and the vector *Macrosteles fascifrons* Stål were used throughout. Hydroponics experiments were performed in a controlled-environment chamber at 27°C with a 16-hour day and an 8-hour night. In other experiments, plants were maintained in a greenhouse at 23° to 27°C.

In our first experiments, small plants of annual chrysanthemum (*Chrysanthemum carinatum* Schousb.) were treated with antibiotic before symptoms appeared. Leafhoppers, after 3 days of feeding for inoculation, were removed from the plants and the roots were washed and treated immediately (or 3 or 6 days later) by immersion for 10 minutes in either chlortetracycline (*6*) or buffer. When plants were repotted in soil, the respective antibiotic or control solution was applied to the foliage in a fine spray (to run-off) at 3-day intervals.

Four separate experiments gave comparable results. Symptoms of aster yellows in plants treated with chlortetracycline at 1000 parts per million (ppm) appeared only when spraying was discontinued (Table 1). Chlortetracycline at 100 ppm also suppressed development of symptoms but some plants, first treated 9 days after exposure to infectious vectors, developed symptoms as soon as did control plants. Penicillin (*7*), on the other hand, had no effect on development of symptoms.

Chloramphenicol (*8*) and tetracycline (*9*) were tested in the same way in five separate trials; all results were comparable (Table 2). Development of symptoms was suppressed by either tetracycline or chloramphenicol, the latter being much less efficacious.

Aster plants having advanced symptoms of aster yellows were then placed in hydroponic culture solution, with or without chlortetracycline (10 ppm). All but one chlortetracycline-treated plant produced new symptomless axillary growth; eventually one plant produced six flower buds that opened to display symptomless flowers (Fig. 1). Control plants developed no new growth during the experiment, and there was no greening of already yellowed leaves.

Suppression of development of symptoms, and remission of existing symptoms of disease, by application of chlortetracycline to foliage, without prior dipping of the roots, was demonstrated in several preliminary experiments with aster, annual chrysanthemum, and celery (*Apium graveolens* L.); treatments were begun within 1 week after appearance of symptoms, so that the plants were much less severely affected by the aster yellows than those used in the

Table 1. Mean incubation periods of aster yellows in plants of annual chrysanthemum treated with chlortetracycline or penicillin. Periods are the mean numbers of days between first exposure to transmitting vectors and the appearance of first symptoms. Each value is based on 24 to 40 plants from four separate experiments. After 3 days of feeding on plants for inoculation, vectors were removed, and the plants were treated either immediately or 3 or 6 days later (day 6 or 9 after first exposure to vectors). Treatment was by immersion of roots in solution for 10 minutes; plants were then replanted in soil. The foliage was subsequently sprayed at 3-day intervals for from 9 to 18 days. Buffer was 0.005*M* phosphate, *p*H 7.5.

First treatment (day)	Period (days) after treatment with			
	Buffer	Chlortetracycline		Penicillin (1000 ppm)
		1000 ppm	100 ppm	
3	20.7	34.9	30.6	21.0
6	17.8	36.1	27.5	15.7
9	18.0	33.4	23.8	17.2

experiments with hydroponics. All parts above ground were sprayed for several weeks with either chlortetracycline (1000 ppm) or control solution containing no antibiotic. Severe symptoms persisted in existing leaves and appeared in new growth of control plants; in plants treated with antibiotic, however, yellowed leaves developed some chlorophyll, and new growth tended to be symptomless. Foliar sprays are much less effective in remission of symptoms than is administration of antibiotic through the roots; adjuvants (see *10*) might improve results with sprays.

In other experiments, chlortetracycline foliar sprays failed to suppress symptoms induced by two well-known viruses: potato yellow dwarf virus in *Nicotiana rustica* L. and wound-tumor virus in *Trifolium incarnatum* L. These results generally agree with previous findings (*11*).

The remission of aster yellows in plants tends to support the tentative hypothesis that the agent of aster yellows is a mycoplasma- or bedsonia-like organism. Confirmation (*12*) of the observation (*1*) of mycoplasma-like bodies in diseased plants and of the presence of such bodies in disease-transmitting but not in nontransmitting vectors (*12*) gives additional support.

Other workers have usually failed to demonstrate inhibition by tetracycline antibiotics of expression of symptoms of plant viruses (*11*), although Leben and Fulton (*13*) reported suppression of symptoms by oxytetracycline. In our preliminary experiments, chlortetracycline did not affect transmission of or symptoms induced in plants by two viruses: clover wound tumor and potato yellow dwarf. Growth of the agent of aster yellows in aster, as determined by bioassay of plant extracts, was completely inhibited (within the sensitivity of the assay) by chlortetracycline administered 3 days after inoculation of the plants. Futhermore, insects acquired aster yellows with greatly reduced efficiency from severely infected aster plants placed 1 week previously in hydroponics with chlortetracycline at 10 ppm. Moreover, we have introduced efficacious antibiotic at many points in the biological cycle of aster yellows without failing to observe suppression of the disease. These results tend to implicate suppression of the disease agent itself.

Table 2. Mean incubation periods of aster yellows in plants of annual chrysanthemum treated with chloramphenicol or tetracycline; each value is based on 30 to 50 plants from five separate experiments. Treatment as for Table 1. Transmitting vectors were left on plants for 2 days of feeding for inoculation. Foliage was sprayed at 2-day intervals for from 4 to 12 days after first treatment by root immersion.

First treatment (day)	Period (days) after treatment with		
	Buffer	Tetracycline (1000 ppm)	Chloramphenicol (1000 ppm)
2	18.1	36.5	23.4
4	18.5	37.4	24.1
6	18.2	33.9	21.6
8	18.0	32.3	21.0

Other properties of the agent, determined by bioassay of infectivity, provide evidence relevant to the etiology of the disease. Sedimentation of the agent of aster yellows in 10 minutes at 30,000*g*, coupled with its passage through a 450-mμ filter, is consistent with the size range of the mycoplasma. Its sensitivity to sonication, chloroform, and surfactants, and its distribution in sucrose-density-gradient zonal centrifugation, provide additional information consistent with mycoplasma- or bedsonia-like etiology.

Nevertheless we recognize that these physical properties and this sensitivity to antibiotics do not eliminate the possibility of an unusual plant virus. The hypothesis that a yellows disease is induced by a mycoplasma- or bedsonia-like organism must be subjected to further scrutiny, including purification from infected tissue, or growth of the causal agent in vitro. If the hypothesis of organism etiology proves to be correct, we shall have to recognize an entirely new class of agents causing disease in plants.

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Fig. 1. Aster plants [*Callistephus chinensis* (L.) Nees] placed in hydroponic culture 4 weeks after inoculation with aster yellows. Plant on left was in the culture with chlortetracycline at 10 ppm for 6 weeks before photography; plant on right was in comparable culture solution for 6 weeks without the antibiotic.

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7. Benzylpenicillinic acid potassium salt.
8. D(—)-Threo-2,2-dichloro-*N*-[β-hydroxy-α(hydroxymethyl)-*p*-nitrophenethyl] acetamide.
9. 4-Dimethylamino-1,4,4a,5,5a,6,11,12a-octahydro-3,6,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-2-naphthacenecarboxamide.
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Proton Magnetic Resonance of Proteins Fully Deuterated except for ^{1}H-Leucine Side Chains

Abstract. *The fully deuterated proteins C-phycocyanin, C-phycoerythrin, and cytochrome c have been obtained by biosynthesis with the leucine side chains, and only the leucine side chains, of normal (^1H) isotopic composition. In these (isotopic hybrid) proteins, proton magnetic resonance analysis shows that the ^1H-leucine side chains are in a variety of environments. During protein biosynthesis, the alpha hydrogen of leucine is exchanged with a hydrogen (^2H) from the aqueous medium.*

There are a number of ways in which fully deuterated proteins can be used for magnetic resonance studies (*1*). We now report results on the incorporation of exogenous (^{1}H)-L-leucine into proteins synthesized by organisms growing in 99.8 percent D_2O (*2*). Algae and photosynthetic bacteria, grown in D_2O with ^{1}H-leucine, synthesized protein in which the leucine amino acid residues that are present contain ordinary hydrogen, and all other nonexchangeable side-chain hydrogen is present as deuterium. We refer to such proteins of mixed isotopic composition as isotopic hybrids. Leucine was chosen for these experiments because it appeared likely that its hydrogen would not appear in other amino acid side chains of the deuterated protein, and this expectation has been confirmed.

The blue-green algae *Phormidium luridum, Fremyella diplosiphon,* and *Synechococcus lividus* (*2*) were cultured in a 99.8 percent D_2O medium to which 0.02 percent of (^{1}H)-L-leucine was added. The cells were harvested, and the proteins C-phycocyanin (and C-phycoerythrin from *Fremyella*) were extracted and purified (*3*). A 50-mg portion of purified protein was then hydrolyzed in 6*N* DCl; the hydrolyzate was taken to dryness, dissolved in D_2O, filtered, dried, and then taken up in 2*N* DCl for proton magnetic resonance analysis (Fig. 1). The (^{1}H)-L-leucine is incorporated into the protein of all three of these algal species, and there is no significant incorporation of protons into other amino acid side chains. The incorporation of leucine into protein occurs with exchange of hydrogen at the α-carbon of leucine with the water of the medium, as no protons attributable to this position are observed in the hydrolyzed protein. From a known concentration of acetate used as an internal standard, we calculate the incorporation of ^{1}H-leucine (as mole percentage of total leucine content of the protein) to be 16 ±5 percent in isotopic hybrid phycocyanin derived from *Phormidium,* 60 ± 15 percent in both phycocyanin and phycoerythrin from *Fremyella,* and 75 ± 15 percent in phycocyanin from *Synechococcus.*

Isotopic hybrid cytochrome c was isolated from *Rhodospirillum* (*4*) grown in D_2O with deuterated algal hydrolyzate (*5*) and added ^{1}H-leucine. In this protein, we found that 80 percent of the leucine was ^{1}H-leucine; this amount was close to the ratio of ^{1}H-leucine to deuterated leucine in the culture medium (*6*). In this case, too, leucine was the only amino acid containing protons which was present in the acid hydrolyzate, and the hydrogen at the α-carbon of leucine had likewise been exchanged by deuterium (*7*).

We have examined native isotopic hybrid deuterated phycoerythrin (molecular weight ~2.3×10^5) containing 60 percent of ^{1}H-leucine by proton magnetic resonance analysis with time-averaging techniques (Fig. 1). The main resonance absorption, arising from the two methyl groups of leucine, is centered at 1.23 ± 0.02 ppm [measured from external hexamethyldisiloxane (HMS)], as compared to 1.26± 0.01 ppm for the center of the methyl lines in monomeric leucine dissolved in D_2O. A considerable fraction of the leucine resonances is observed at higher field, centering at about 0.6 ppm. Thus, the proton magnetic resonance data

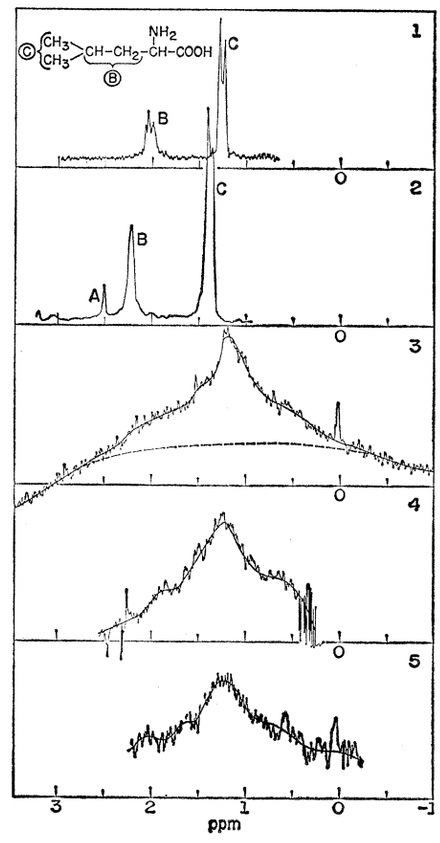

Fig. 1. *Spectrum 1.* The upfield portion of the proton magnetic resonance (PMR) spectrum of ^{1}H-leucine, 1 percent in D_2O-sodium phosphate buffer, 0.01*M*, *p*D 7.5. *Spectrum 2.* The upfield portion of the PMR spectrum of acid-hydrolyzed phycoerythrin substituted with ^{1}H-leucine. The sample (7 percent amino acids) is dissolved in 2*N* DCl, so the leucine lines are shifted downfield. Peak A is internal acetate, 0.0042*M*; accumulated scans, 67. *Spectrum 3.* A 1.7 percent solution of leucine-substituted deuterated phycoerythrin in D_2O-phosphate buffer; accumulated scans, 882; sweep rate, 2 cycle/sec^2. The dashed line is our estimate of the position of the base line. *Spectrum 4.* Solution as in spectrum 3; accumulated scans, 489; sweep rate, 5 cycle/sec^2. *Spectrum 5.* An 0.8 percent solution of leucine-substituted deuterated phycoerythrin in D_2O-phosphate buffer; accumulated scans, 969; sweep rate, 5 cycle/sec^2. The relatively sharp line at 0.6 ppm is probably real and associated with the protein. The line near 0 ppm in both spectra 3 and 5 is an artifact, perhaps due to a trace of silicone. All chemical shifts are given from external hexamethyldisiloxane. The protein spectra were recorded with external benzene to provide the field-frequency lock. Probe temperature, 32°C.