virtually 100 percent probability of manifesting IIS's when challenged with elevated K^+ alone; fewer PTZ injections reduced this probability in a dose-dependent manner (Fig. 2A). Second, in animals given five PTZ injections, the K^+-induced epileptiform discharge persisted for at least 20 days (Fig. 2B) after the last injection (*13*). In rodents, less than 5 percent of an injected dose of PTZ remains in the brain after 24 hours (*14*). Furthermore, no spontaneous convulsions were observed after termination of the injections, suggesting that there was not a prolonged drug exposure at convulsant levels. However, this is, at best, indirect evidence that does not entirely preclude the presence of some small level of compartmentalized PTZ in the tissue. To test this possibility, we placed enough PTZ (5 to 10 μg/ml) in the superfusion medium of control slices to generate IIS directly, and recorded the washout time. Enough PTZ could be washed out of the slice in 1 hour to terminate spontaneous spiking. Together with the previously cited data, this result makes it seem unlikely that residual PTZ is responsible for the heightened excitability found in kindled slices.

In conclusion, our data show that hippocampal slices from kindled animals no longer require a chemical convulsant in the medium to trigger epileptiform activity when the concentration of K^+ is elevated. Since the sensitivity to elevated K^+, modulatory influences of divalent cations, and responses to electrical tetanization are similar to those seen in slices from control animals, we hypothesize that kindling induces a change in basic membrane or synaptic properties similar to that induced acutely by convulsants such as penicillin. In view of the postulated relation between kindling and epilepsy (*15*) and enduring neuropsychological changes such as learning and memory (*16*), future research employing the model described in this report may provide important insights into normal and abnormal neuronal function.

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9. The IIS's recorded here, consisting of biphasic bioelectric potentials of large amplitude and long duration, are extracellular manifestations of paroxysmal depolarizing shifts. This correlation was well established in epilepsy models in vivo [D. A. Prince, *Exp. Neurol.* **21**, 307 (1968); M. A. Dichter and W. A. J. Spencer, *Neurophysiology (USSR)* **32**, 663 (1969)], and in hippocampal slices in vitro [A. P. Oliver, B. J. Hoffer, R. J. Wyatt, *Epilepsia* **18**, 543 (1977); A. P. Oliver *et al.* (*8*); A. P. Oliver, J. S. Carman, B. J. Hoffer, R. J. Wyatt, *Exp. Neurol.*, in press].
10. The kindling procedure with PTZ is similar to that used in other laboratories [T. Ito, M. Hori, K. Yoshida, M. Shimizu, *Eur. J. Pharmacol.* **45**, 165 (1977); S. T. Mason and M. E. Corcoran, *Brain Res.* **170**, 497 (1979)].
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12. Each slice was about 350 μm thick. Details of our techniques have been described (*9*). The basic medium for superfusion had the following composition (millimoles per liter): NaCl, 124; KCl, 5; $CaCl_2$, 2.4; $MgSO_4$, 1.3; KH_2PO_4, 1.24; glucose, 10; and $NaHCO_3$, 26. Sodium penicillin G (Sigma) or concentrated K^+ or both were added to the basic perfusion medium in some experiments. All recordings were obtained from the CA_3 region of the slice with a glass micropipette filled with 3*M* NaCl (impedance, 1 to 5 megohms), amplified by conventional means, and displayed on an oscilloscope and a strip-chart recorder. The IIS frequency was counted directly from the strip-chart record. Electrical stimulation of the fimbria was performed with 10 to 20 repetitive stimuli per second. Each stimulus was a 10-V monophasic square wave lasting 50 μsec.
13. An obvious question in the kindling paradigm concerns possible disruption of the hippocampal inhibitory interneurons secondary to post-seizure hypoxia. Selective vulnerability of inhibitory interneurons to hypoxia has been reported for the spinal cord [R. A. Davidoff, L. T. Graham, R. P. Shank, R. Werman, M. Aprison, *J. Neurochem.* **14**, 1025 (1967)] and the hippocampus [T. Dunwiddie, A. Mueller, M. Palmer, J. Stewart, B. Hoffer, *Brain Res.*, in press]. Several observations suggest that this problem did not occur in our experiments. First, most animals studied physiologically manifested hyperpnea during and after PTZ-induced seizures, an effect well documented in the literature. Second, no spontaneous seizures were observed between the last kindling trial and physiological testing of the slices. Finally, action potential discharge in single pyramidal neurons in kindled slices, prior to elevating the K^+ concentration, resembled that seen in normal slices, again suggesting intact inhibitory mechanisms.
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Cloned Cauliflower Mosaic Virus DNA Infects Turnips (*Brassica rapa*)

Abstract. *Cauliflower mosaic virus DNA cloned in the Sal I site of bacterial plasmid pBR322 infects turnip plants. The cloned viral DNA must be excised from the recombinant plasmid to infect, but need not be circularized and ligated in vitro. The cloned viral DNA lacks site-specific single-strand breaks found in DNA obtained directly from the virus. However, these breaks are reintroduced into the viral genome during multiplication of the virus in the plant host.*

Cauliflower mosaic virus (CaMV) has attracted attention in the past few years because of its possible use in plant "genetic engineering" studies. Cauliflower mosaic virus is one of the few plant viruses containing double-strand DNA, and the DNA, when isolated from the virus, can infect plants (*1*). These properties have led to the suggestion that the CaMV DNA could be used as a vehicle for introducing foreign DNA in plant cells (*2*) in much the same fashion as SV40 viral DNA has been used to introduce DNA in animal cells (*3*). Since the construction of a CaMV DNA vehicle would involve recombinant DNA technology, it is important to show first that cloned CaMV DNA can infect plants.

Szeto *et al.* (*2*) dampened much of the optimism for using CaMV DNA as a vehicle in plants. They found that plants could not be infected by cloned CaMV DNA or by CaMV DNA cleaved at a single site by a restriction enzyme and religated in vitro. They attributed the failure to one of several factors, including the possibility that some secondary structure of the DNA, crucial for infectivity, was lost when the viral DNA was cleaved (*2*).

Since the report by Szeto *et al.* (*2*), several studies have shown that, indeed, the CaMV genome has an unusual secondary structure. The genome isolated from the virus is found in both linear and open (noncovalently closed) circular forms (*4*) with two or three (depending

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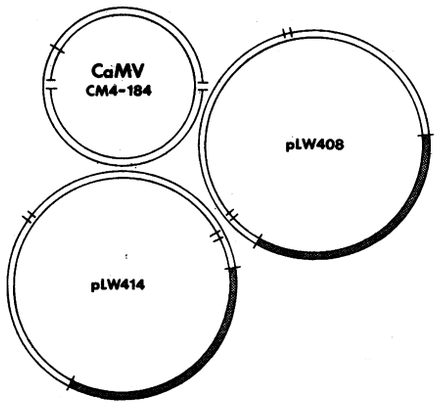

Fig. 1. Structure of CM4-184 CaMV DNA and recombinant plasmids pLW408 and pLW414. CaMV DNA was cleaved at the single Sal I site (|) and inserted in the Sal I site of pBR322 (crosshatched sector). The CM4-184 CaMV DNA has two naturally occurring single-strand breaks (—||—), which are closed (—††—) during cloning of the recombinant plasmid.

on the strain) site-specific single-strand breaks (*5*, *6*). Despite the presence of these breaks, the circular DNA, for reasons which are not yet understood, is found in a mixture of twisted or tangled forms (*7*).

We attempted to repeat the infectivity experiments of Szeto *et al.* (*2*) to see if we could find conditions for successfully infecting plants with cloned CaMV DNA. We cloned the entire CaMV genome [from viral isolate CM4-184, originally designated CM1841 (*8*)] by cutting the DNA at the single Sal I site and inserting it into the Sal I site in pBR322 (Fig. 1). We obtained clones representing the insertion of CaMV DNA in the plasmid in both orientations. DNA from the viral isolate CM4-184 (*5*) has two site-specific single-strand breaks, one in each strand and nearly opposite each other across the CaMV DNA circle (Fig. 1). These breaks were sealed during cloning as evidenced by the fact that the recombinant plasmids bearing CaMV DNA behaved on CsCl-ethidium bromide density gradients as covalently closed circular DNA forming a single band at about 1.59 g/cm^3.

We tested whether CaMV DNA obtained from these recombinant plasmids could infect turnip plants (*Brassica rapa* L., cultivar Just Right). Because turnip is not a local lesion host for the virus under normal conditions, infection was scored by the appearance of systemic symptoms—vein clearing and leaf wrinkling. In control experiments we found that DNA isolated directly from the virus is infective either in an intact form or when cleaved at a single site by Sal I (Table 1). This contrasts to the report by Szeto *et al.* (*2*) which showed that Sal I cleavage destroys infectivity of viral DNA.

Table 1. Infectivity of CaMV DNA derived from recombinant plasmids and from virus particles. Inoculum (50 μl) contained DNA, as indicated, in 150 m*M* NaCl, 15 m*M* sodium citrate, and 2 mg of Celite 545 (an abrasive). The inoculum was rubbed on the leaves of two or three 2-week-old turnip plants. Plants were scored for the appearance of symptoms 4 to 5 weeks after infection.

Inoculum	Plant infected/ plant inoculated
Mock infected (no DNA)	0/3
CM4-184 CaMV (10 μg)	3/3
CM4-184 CaMV/Sal I* (10 μg)	2/3
pLW408 (20 μg)	0/3
pLW408/Sal I* (1 μg)	0/2
pLW408/Sal I* (5 μg)	1/3
pLW408/Sal I* (20 μg)	3/3; 2/2
pLW414 (20 μg)	0/2
pLW414/Sal I* (20 μg)	2/2
pLW408/Sal I, nuclease S1 digested† (20 μg)	0/2

*Sal I digested 3 hours with 0.5 enzyme unit per microgram of DNA. †Nuclease S1 digested 30 minutes with four enzyme units per microgram of DNA.

The Sal I-digested recombinant plasmid, pLW408 or pLW414, which contains the CaMV genome, can also infect plants (Table 1). The cloned CaMV DNA is infective only when it is excised from the plasmid. The intact plasmid is not infective. The cloned CaMV DNA lacks the normal secondary structure of the viral genome, including the single-strand breaks and the tangled form, and therefore these structural features must not be required for infectivity. In addition, the viral DNA, whether derived from the plasmid or directly from the virus, does not need to be circular to infect plants. The plant host is apparently capable of circularizing the DNA as long as the cohesive ends of the Sal I remain intact. Mild nuclease S1 digestion of the Sal I-cleaved pLW408 plasmid (pLW408/Sal I), a treatment which should digest single-stranded DNA at the cohesive ends, destroys infectivity of the plasmid (Table 1).

We investigated whether the appearance of symptoms in the plants infected with cloned viral DNA was accompanied by virus production. We passaged the infective agent from a plant infected with Sal I-digested cloned DNA to a batch of plants from which virus could be extract-

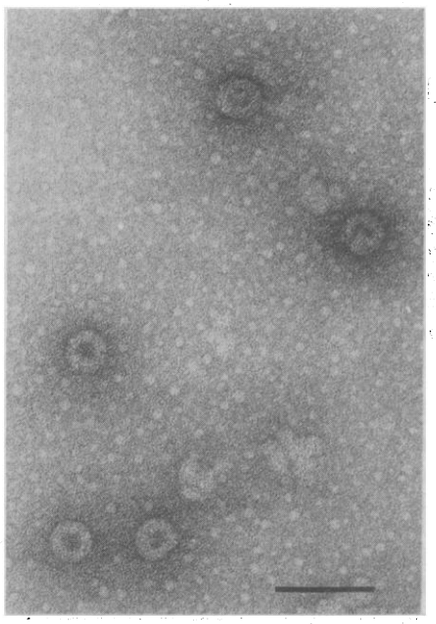

Fig. 2 (left). Electron micrograph of virus particles obtained from infection with pLW414/-Sal I. Virus particles were isolated (*9*) and stained with uranyl acetate. Scale bar, 100 nm.

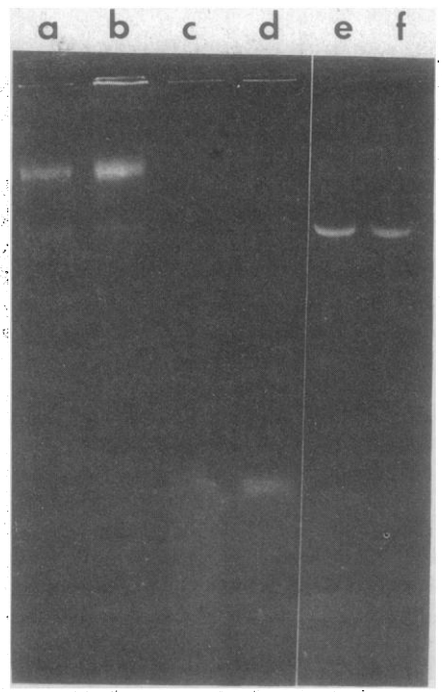

Fig. 3 (right). Agarose gel electrophoresis migration behavior of DNA obtained from the standard virus and DNA from particles resulting from infection by pLW408/Sal I. DNA in 1 percent agarose gels in 40 m*M* tris acetate (*p*H 7.9), 20 m*M* sodium acetate, and 1 m*M* EDTA was stained with ethidium bromide (1.5 μg/ml) and photographed with ultraviolet illumination. (a) DNA (2 μg) from the standard CM4-184 virus in native form, (c) following heat denaturation at 100°C for 2 minutes and quick cooling, and (e) after digestion with 4 units of Sal I for 2 hours. (b) DNA from particles resulting from infection with pLW408/Sal I in native form, (d) following heat denaturation and quick cooling, and (f) after Sal I digestion.

ed. Virus extraction procedures (*9*) yielded 50-nm spherical particles identical in morphology to standard CaMV (Fig. 2).

To determine whether the virus particles contain DNA characteristic of the original virus, we extracted the nucleic acid and subjected it to electrophoretic analysis on agarose gels. DNA from the particles comigrated with DNA from the standard virus, both in native and denatured form (Fig. 3). The native DNA's migrated as two bands, a faster sharp band composed of linear molecules and a slower diffuse band composed of a collection of circular forms (*7*). The denatured DNA from the standard CM4-184 virus contains two single-strand breaks (Fig. 1), and when it is denatured it falls apart into two linear single strands that comigrate as a single band on gels (*5*). The gel migration properties of the native and denatured DNA indicate that viral DNA molecules derived from the infection by cloned CaMV DNA have reacquired their secondary structure and their single-strand breaks. The Sal I digestion of the DNA obtained from virus particles isolated from plants infected with cloned CaMV DNA shows that the plant rejoined the Sal I cohesive ends of pLW408/Sal I DNA, thereby reconstituting the Sal I site.

It should be pointed out that the cloned CaMV DNA suffered no major insertions or deletions during reintroduction into the plant. The DNA retained all characteristics of the CM4-184 CaMV DNA from which it was derived, including the DNA fragment pattern resulting from the digestion with Sal I (Fig. 3) and several other restriction enzymes (data not shown). This is important because it means that the virus particles that arose from infection by cloned CaMV DNA did not, in fact, result from the accidental introduction of another strain of CaMV. Because CM4-184 DNA carries a large deletion, amounting to 5 percent of the total genome (*5*), it is clearly distinguishable from other CaMV types. The CM4-184 strain is unlikely to be accidentally introduced into our plants because the virus by itself cannot be transmitted by aphids, the natural vector for the virus (*8*). We routinely maintain another viral isolate, CaMV Cabb B-J.I. (an aphid-transmitted strain), in the same greenhouse with the test plants. The DNA from this strain can be clearly distinguished from CM4-184 DNA by gel electrophoretic analysis.

These experiments demonstrate that DNA obtained from plasmids constructed in vitro can infect plants and thereby open new opportunities for exploring the use of CaMV as a molecular vehicle in plants.

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Mitochondrial Water in Myocardial Ischemia: Investigation with Nuclear Magnetic Resonance

Abstract. *Nuclear magnetic resonance studies of mitochondria isolated from ischemic hearts after coronary vessel occlusion indicated a decrease in water proton relaxation times. This change coincided with a decrease in the hydration of the samples. It is suggested that in ischemia, changes in macromolecular hydration may be one of the first mechanisms to alter function in the mitochondria, which are vital to the energy-transducing process in heart muscle.*

Myocardial ischemia leads to rapid deterioration of mitochondrial function (*1-3*). This occurs even during the first 20 minutes of coronary vessel occlusion (the "reversible" stage). Irreversible cellular damage after 20 minutes of occlusion has been attributed to several factors (*4-8*). However, no conclusive evidence has been presented to account for the very early and progressive deterioration in organelle function that occurs with ischemia.

Nuclear magnetic resonance (NMR) studies have indicated the importance of cellular water and its exchange for several biological systems in normal and certain pathological states (*9-11*). In light of morphological evidence that mitochondria become swollen and ultimately disrupted during ischemia (*12-14*), we chose to ascertain whether changes in macromolecular hydration are associated with the altered function. Mitochondrial water content was investigated by measuring the NMR relaxation times of water protons. We report that the hydration of mitochondrial pellets and the spin-lattice (T_1) and spin-spin (T_2) relaxation times of water protons decrease as the period of ischemia increases. In fact, these changes begin to appear after just 3 minutes of coronary occlusion.

A loose ligature was placed on the circumflex coronary artery of surgically prepared and monitored dogs (15 to 25 kg) (*13*). The ligature was tightened until flow ceased entirely, and the heart was removed after a set period and immediately chilled to 4°C. Control animals received sham surgery; no occlusion was performed and the heart was removed after periods as long as 60 minutes. Mitochondria were isolated from 10- to 15-g samples of both the anterior and posterior papillary muscle regions of the left ventricle by using the Polytron procedure with either KCl-EDTA (*15*) or mannitol; additionally, two populations of mitochondria were isolated by the Polytron and Nagarse protocol of Palmer *et al.* (*16*). Protein was measured by the biuret method (*17*).

The mitochondrial suspension was diluted to 20 mg/ml with isolation medium, and conical plastic NMR tubes were filled with 0.45 ml of the suspension and centrifuged at 10,000*g* for 10 minutes. The supernatant fraction was aspirated, and any liquid adhering to the inside of the tube was absorbed with a cotton

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