per. The geological structures allow the association of the two pieces.

The isotopic ratios are moderately close: the difference is 0.4 per mil for $\delta^{13}C$ and 0.3 per mil for $\delta^{18}O$. The isotopic ratios are diagnostic for Pentelic marble and allow the association of the stones (*11*).

EM 2685 (IG II², 185)/EM 6958 (IG II², 121). Geological features in both inscriptions are similar. Prominent fold axes on the backs run from top to bottom, parallel to the lettering (Fig. 3). The grain size in both is about ½ to 1 mm; accessory micas are present. Sample EM 2685 is white to dark gray, and EM 6958 is white to yellowish white. Because these colors have been affected by weathering, we cannot know how much credence to give to this criterion.

Although the geological features allow an association, the isotopic ratios are sufficiently different that it is impossible for these stones ever to have been part of the same stele. The difference is 1.2 per mil in $\delta^{13}C$ and 2.4 per mil in $\delta^{18}O$. The isotopic values suggest that EM 2685 came from quarries on Mount Hymettus and EM 6958 from Mount Pentelikon (*12*).

EM 12892/EM 13393. Geological features of the two are similar. Foliation is very weak and appears to be almost flat, dipping about 10° or less to the bottom. Mica is abundant in both and forms a lineation, as seen on the face that runs from top to bottom. The grain size is generally ¼ to ½ mm but reaches a maximum of 1 mm. Both pieces are white to gray in color, but EM 12892 shows more severe weathering effects. The isotopic data do not allow the inscriptions to be associated (*13*).

Conclusions. In many of the inscriptions examined, we discovered that the ancient quarrymen took advantage of the fact that marble tends to split most easily along its foliation planes as well as along its more prominent lineations. Thus any inscription with a foliation plane parallel to its face and a lineation perpendicular to the lettering direction, running from top to bottom, was oriented that way by the quarryman for ease of cutting. Most of the stelai have the same orientation; all that can be said about two separate inscriptions with similar foliation and lineation features is that their geological structures permit an association. Other geological features such as color, banding, grain size, and accessory minerals should also be examined. If all geological features match, the isotopic ratios of the inscriptions should be determined before they are associated as parts of the same original stele.

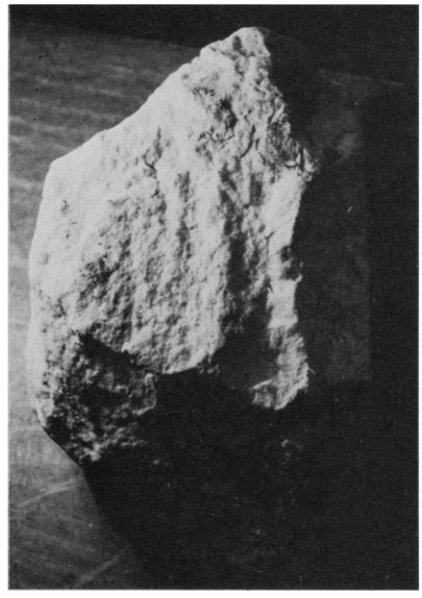

Fig. 3. Sample EM 6958. A strong lineation by fold axes that run from top to bottom and parallel to the direction of lettering on the face is seen on the back. The stone is 22 cm wide.

In any particular stele, the variation in $\delta^{13}C$ and $\delta^{18}O$ values should be less than about 0.4 per mil. If the differences are greater, the stones could not have been part of the same original. We recommend that whenever there is doubt about the association of stones, both a detailed examination of the geological features and an isotopic analysis be carried out. An association is justified only if both agree. The techniques described here as well as our conclusions are equally applicable to fragmentary marble sculpture.

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References and Notes

1. G. R. Lepsius, *Griechische Marmorstudien* (Preussische Akademie der Wissenschaften, Berlin, 1890); C. Renfrew and J. S. Peacey, *Annu. Br. Sch. Athens* **63**, 45 (1968); B. Ashmole, *ibid.* **65**, 1 (1970).
2. N. Herz and W. K. Pritchett, *Am. J. Archaeol.* **57**, 71 (1953).
3. H. Craig and V. Craig, *Science* **176**, 401 (1972).
4. N. Herz and D. B. Wenner, *Geol. Soc. Am. Abstr. Programs* (1977), vol. 9; ———, E. J. Walters, *Am. Inst. Archaeol. Abstr. 79th Gen. Meet., Athens, Ga.* (1977), p. 39.
5. S. M. F. Sheppard and H. P. Schwarcz, *Contrib. Mineral. Petrol.* **26**, 161 (1970).
6. H. Craig, personal communication.
7. The two pieces were tentatively associated by W. K. Pritchett [*Calif. Stud. Classical Antiq.* **5**, 169 (1972)].
8. *Athenian Agora* **15** (No. 46) (1974).
9. D. M. Lewis [*Annu. Br. Sch. Athens* **50**, 26 (1955)] correctly identified the first two pieces as being from the same inscription, but from different blocks.
10. Sample EM 12910 was associated with the other two pieces (IG II², 1654) by E. Schweigert [*Hesperia* **7**, 268 (1938)]. The association was rejected by W. E. Thompson [*ibid.* **39**, 56 (1970)], primarily on the basis of the spacing of the letters.
11. These two stones, which, according to P. Peppas-Delmouzou, director of the Epigraphical Museum, were found together and identified by workers in marble at the National Archaeological Museum of Greece as being from the same stele, were associated by M. T. Mitsos [*Archaeol. Ephimeris* (1965); p. 134]. Later, O. W. Reinmuth [*The Ephebic Inscriptions of the Fourth Century, B.C.* (Leiden, 1971), pp. 1–4] referred to them as "the earliest known ephebic inscription." In favor of the disassociation of the two pieces are A. G. Woodhead, [*Suppl. Epigr. Graecum* **78**, 23 (1968)], D. Lewis [*Classical Rev.* **23**, 254 (1973)], and (at great length) F. W. Mitchel [*Z. Papyrol. Epigr.* **19**, 233 (1975)]. Mitsos replied to his critics in *Archaeol. Ephimeris Chron.* (1975), p. 39.
12. A. Wilhelm's association of the two pieces was communicated to J. Kirchner, who recorded Wilhelm's opinion in his commentary to IG II² 185. M. J. Osborne [*Annu. Br. Sch. Athens* **66**, 323 (1971)] rejected the association.
13. The two stones from the north slope of the Acropolis were tentatively associated by R. S. Stroud [*Hesperia* **40**, 174 (1971)].
14. The original idea for this study came from W. K. Pritchett, who also supplied the epigraphic references. Procedures and conclusions are our own responsibility. We thank J. E. Noakes and B. K. Sen Gupta for critically reviewing the manuscript. We acknowledge support from the American Philosophical Society, Johnson Fund grant 1270 (to N.H.), and NSF grant DES 74-13268 (to D.B.W.). We are also indebted to P. Peppas-Delmouzou for her kind cooperation and assistance.

26 September 1977; revised 5 December 1977

Rabies Viruses Increase in Virulence When Propagated in Neuroblastoma Cell Culture

Abstract. *Several strains of attenuated rabies virus lacking the capacity to kill adult mice acquired a high lethal potential for mice after one to five serial passages in murine or human neuroblastoma cells. The virulence acquired after passage in neuroblastoma cells is a stable genetic trait retained during subsequent passage of viruses in nonneuroblastoma cell systems.*

Myriad types of animal-pathogenic viruses, including rabies virus, have been reduced in virulence by passage in cell cultures. Diverse cell culture systems, often selected serendipitously, have been used, and the attenuation obtained has usually required many serial passages. Flury high egg passage (HEP) rabies virus, widely used as a live virus veterinary vaccine, was attenuated, as indicated by loss of lethal potential for adult mice, after 174 passages in chick embryos (*1*). This virus regained pathogenic potential for adult mice after passage in newborn mice (*1*) but has never been reported to revert to virulence after passage in cell culture.

Enhancement of virus virulence after

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cultivation in cells in vitro at physiological temperatures has rarely been reported (*2*). However, reversion of attenuated viruses to the virulent state has been demonstrated in infected animals (*3*). The potential for possible reversion to virulence is a constant theoretical concern of all advocates of live virus vaccines. Unfortunately, the complex environment of the living animal renders the analysis of factors favoring emergence of revertant virulent virus extremely difficult.

We now describe the consistent enhancement of virulence of Flury HEP and certain other attenuated strains of rabies virus after a limited number of virus passages in vitro in murine or human neuroblastoma cell cultures.

Mouse neuroblastoma C1300 cells share with neurons the following characteristics: gross microscopic (*4*) and fine-structural neuronlike morphology (*5*); the presence of microtubular protein (*4*), protein 14-3-2 (*6*), and neurotransmitter synthetic enzymes (*7*); and electrically excitable cell membranes (*8*) with acetylcholine receptors (*9*). Clone NA, used in our studies, is a hypoxanthine-guanine phosphoribosyl transferase (HGPRT)-deficient clone derived from C1300 cells (*10*).

The attenuated rabies viruses used in these studies were two separate substrains of Flury HEP, one adapted to growth in BHK cells and one to chick fibroblasts (CF); Kelev, a strain isolated in Israel and serially passaged in chick embryos (*11*); and three avirulent variant CVS clones, S_1, S_2, and S_3 [small plaque variants selected from normally highly mouse-virulent BHK/21 cell-adapted CVS (*12*) rabies virus (*13*)]. These six viruses were serially passaged in NA cells. For comparative purposes, the same viruses were passed in nonneuroblastoma cell lines or in vivo in intracerebrally inoculated newborn mice (Table 1). After viruses were passed in experimental host systems, the lethal efficiency of each virus [adult mouse 50 percent lethal doses per plaque forming unit (LD_{50}/PFU)] was compared with its infective efficiency [infective doses (ID_{50}/PFU)]. The infective efficiency was determined by challenging animals that survived the initial experimental virus titration with a standard dose (30 to 50 LD_{50}) of lethal challenge virus (strain CVS). Survivors of this challenge were considered to have been infected.

Each of the six attenuated viruses was determined to be virtually free of lethal potential for adult mice prior to experimental passage, although each virus efficiently infected mice [that is, the minimum infectious efficiency (CVS-S3) was 0.012 ID_{50}/PFU, or the mean dose required for protection was 83 PFU]. Each of the six test viruses acquired an efficient mouse-lethal capability after from two to five passages in NA cells. Each virus caused some mouse mortality after a single passage in NA cells, with the lethal efficiency increasing rapidly after subsequent NA cell passage. Determination of lethal end points of viruses with a limited lethal potential after early NA cell passage was often impossible because such viruses frequently exhibited abnormal dose-lethal response curves that suggested "autointerference" at high dosage [for example, HEP Flury CF and NA (third passage); other data are not shown] (*14*).

The acquisition of lethal potential often, but not always, occurred less rapidly during passage in NA cells than during passage in vivo in brains of suckling mice. No acquisition of virulence was detected during serial passage of the same viruses in two substrains of BHK cells, or in human fibroblasts (WI-38) or in cells of a cell line derived from cow brains [CB3 (*15*)].

The lethal factor recovered from virus preparations passed in NA cells was rabies virus and not a contaminating latent agent of NA cells. The lethal potential of six different virus preparations

Table 1. Effect of passing attenuated rabies viruses in clone NA mouse neuroblastoma cells, nonneuroblastoma cells, and suckling mouse brain in vivo on virulence. Cell cultures were infected initially at a virus multiplicity of infection of 1.0 to 10.0 and incubated at 35°C for 3 to 7 days, depending on the severity of cytopathic effect. Serial passages were performed with undiluted cell supernatants. Suckling mice were inoculated intracerebrally with virus doses of 10^5 to 10^6 PFU and killed when moribund; a mouse brain suspension (diluted 1 : 10) was used for serial passage. Virus harvests were titrated for their content of plaque-forming units in BHK-13s cells suspended in agarose (*24*) and for mouse LD_{50} in 4-week-old ICR random-bred mice inoculated intracerebrally. The mouse infectious dose (ID_{50}) content was determined by challenge of surviving mice at 30 days after infection with 30 to 50 LD_{50}'s of CVS rabies virus inoculated intracerebrally.

Virus	Experimental passage level		LD_{50}/PFU	ID_{50}/PFU	Relative virulence (LD_{50}/ID_{50})
	Cells	Passages			
HEP Flury (BHK)	Stock	None	<0.00000017	0.49	<0.0000034
	NA	2	0.060	0.43	0.14
	BHK/21	5	<0.00000055		
	BHK-13S	5	<0.0000021		
	CB3	3			
	+	+	<0.00096		
	BHK	6			
	WI38	6			
	+	+	<0.00000044		
	BHK	5			
	smb*	1	0.98	0.98	1.0
HEP Flury (CF)	Stock	None	<0.0000020	0.067	<0.000030
	NA	3	0.0019	0.046	0.042
	BHK/21	5	<0.00000035		
	BHK-13S	5	<0.000000075		
	smb*	4	0.00050	5.5	0.00091
Kelev	Stock	None	<0.00019	2.5	<0.000075
	NA	5	0.095	0.34	0.28
	BHK	5	<0.00000052		
	smb*	4	0.039	0.39	0.10
CVS-S1	Stock	None	<0.000000037	0.22	<0.00000017
	NA	2	0.018	0.083	0.21
	BHK	5	<0.000015		
	CB3	3			
	+	+	<0.000033		
	BHK	5			
	WI38	6			
	+	+	<0.00000039		
	BHK	5			
	smb*	2	0.13	0.17	0.73
CVS-S2	Stock	None	<0.0000013	0.10	<0.000013
	NA	4	0.0071	0.016	0.43
	BHK	5	<0.0000075		
	smb*	2	0.23	0.46	0.50
CVS-S3	Stock	None	<0.000013	0.012	<0.0011
	NA	4	0.0048	0.11	0.042
	BHK	5	<0.0000039		

*Suckling mouse brain.

passed in neuroblastoma cells was shown to be completely neutralized after incubation with rabbit antiserums prepared against purified rabies virions (data not shown).

The observed enhancement of rabies virus virulence after cultivation in NA cells might have been attributable either to the neuronlike properties of the cells or to adaptation to mouse cells. NA cells are the only cells of murine origin in which we can now cultivate rabies virus to high titer. As a test of these alternatives, the Flury HEP and CVS-S1 strains of attenuated rabies virus were serially propagated in cells of three human neuroblastoma cell lines, IMR-32 (*16*), SK-N-SH (*17*), and SK-N-MC (*17*) by methods identical to those used with NA cells. Rabies virus readily replicated to high titer in each human neuroblastoma cell line with no requirement for prior adaptation. The effect of these passages on virulence for mice is shown in Table 2. Both Flury HEP and CVS-S1 viruses acquired a high degree of lethal potential for adult mice within two cell passages in either cell line IMR-32 or SK-N-SH. Virulence was acquired with a rapidity equal to or greater than that observed after two passages in murine NA cells. However, no lethal potential was acquired by either attenuated virus after three serial passages in SK-N-MC cells. These observations suggested that neuroblastoma cell characteristics, rather than mouse cell characteristics, lead to enhancement of virulence in attenuated rabies virus. We performed six serial passages in BHK/21 cells of HEP Flury and CVS-S1 viruses that had regained mouse virulence after either two passages in NA cells or one or two passages in brains of newborn mice. Each of these viruses retained full virulence for adult mice (LD_{50}/ID_{50} = 1.0) after this extended BHK/21 cell passage. The observation suggests that genotypically altered virulent virus is selectively replicated in neuroblastoma or brain cells. The possibility of increased virulence as a result of possible physical incorporation of neuronal cell components into virions cannot be substantiated by these results.

Several investigators have induced the selective replication of virulent enteroviruses in cell cultures inoculated with attenuated virus and incubated at supraphysiological temperatures (*18*). This approach was based on the partial correlation observed between virulence and an enhanced capacity to replicate at high (40°C) temperature noted in several studies of polioviruses (*19*) but not observed in our studies of rabies virus (*20*). We have not found an increase in reproductive capacity at 40°C in Flury HEP or CVS-S1 viruses rendered virulent by passage in NA, IMR-32, or SK-N-SH cells or in newborn mouse brain (data not shown). Furthermore, we have demonstrated that both attenuated Flury HEP and CVS-S1 viruses acquire mouse virulence after serial passage at the subphysiological incubation temperature of 30°C in NA or IMR-32 cells (Fig. 1). Acquisition of virulence in cells incubated at this temperature appeared to proceed slightly more slowly than at normal incubation temperatures of 35° to 36°C, but virulence for mice was detected after three passages in each system.

The neuroblastoma cell culture systems offer a unique means of studying host cell factors influencing virus virulence, uncomplicated by systemic defensive responses such as humoral and cell-mediated immune response, hyperthermic response, and endocrine hormone influences, operative in vivo. A survey of only four neuroblastoma cell types has revealed differences in their ability to favor replication of rabies virus of enhanced virulence, despite the fact that each cell type expresses certain neuronlike phenotypic characters. Inasmuch as a number of biochemical and physiological markers distinguishing the three human neuroblastoma cell lines described above have been reported (*21*), and markers distinguishing separate clones of C1300 mouse neuroblastoma cells have also been characterized (*22*), it may be possible to identify certain cell properties associated with enhancement of virus virulence. Whether the observed enhancement of virus virulence represents a selective replication of randomly occurring back mutants to virulence or some other possible mechanism remains to be determined.

These neuroblastoma cell systems also provide a convenient tool for developing

Table 2. Effect on virulence of passage of attenuated rabies viruses in human neuroblastoma cell lines. Methods are the same as those described for Table 1.

Virus	Experimental passage level		LD_{50}/PFU	ID_{50}/PFU	Relative virulence (LD_{50}/ID_{50})
	Cells	Passages			
HEP Flury (BHK)	Stock	None	<0.00000017	0.49	<0.0000034
	IMR-32	2	0.088	0.13	0.67
	SK-N-SH	2	0.085	0.19	0.45
	SK-N-MC	3	<0.0000029	0.29	<0.000010
CVS-S1	Stock	None	<0.000000037	0.22	<0.00000017
	IMR-32	2	0.12	0.12	1.0
	SK-N-SH	2	1.0	1.0	1.0
	SK-N-MC	3	<0.0000050	0.11	<0.000045

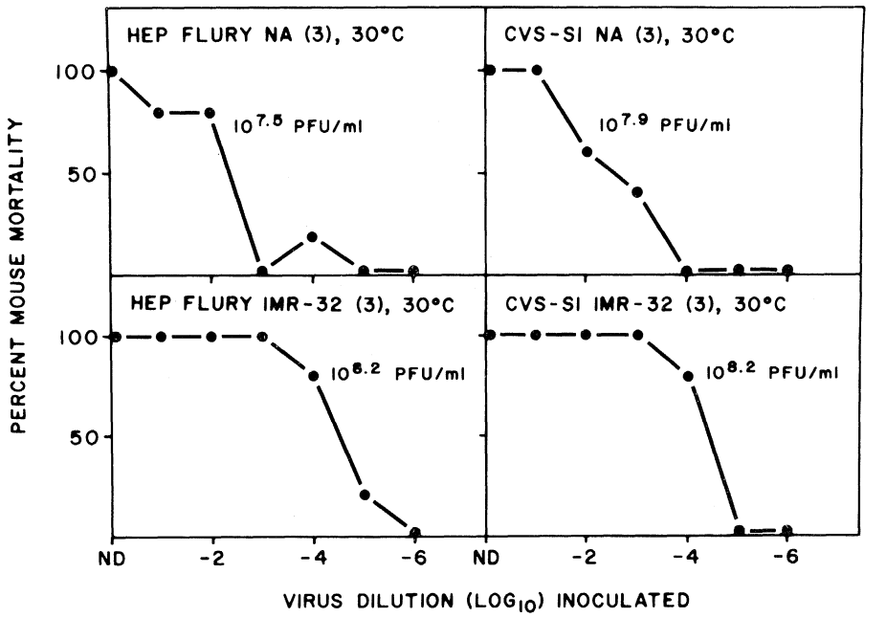

Fig. 1. Lethal potential, in 4-week-old ICR mice, of attenuated rabies viruses passaged three times in NA or IMR-32 neuroblastoma cells incubated at 30°C.

virulent and avirulent sets of rabies viruses of identical genetic background separated by a minimal number of replication generations. Such sets of viruses may allow a more fruitful search for virus markers associated with virulence or attenuation than have the previously used systems of attenuated viruses compared to virulent ancestors from which they were derived usually by prolonged passage in "unnatural" host systems.

Finally, the mechanism of death induced by rabies virus is incompletely understood, although rabies virus replication is known to occur preferentially in neurons within the central nervous system (*23*). Since neuroblastoma cell lines exhibit various neuronlike physiological and biochemical characteristics, a study of the effect of rabies virus infection on the expression of these neuronal characters in vitro may shed light on the mechanism of rabies virus pathogenesis occurring at the cellular level in vivo.

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References and Notes

1. H. Koprowski, *Bull. W.H.O.* **10**, 709 (1954).
2. Foot and mouth disease virus [R. Maes, *Arch. Gesamte Virusforsch.* **29**, 63 (1970)] and influenza virus [H. T. Maasab, T. Francis, F. M. Davenport, A. V. Hennessy, E. Minuse, G. Anderson, *Bull. W.H.O.* **41**, 589 (1969)] attenuated mutants have been reported to regain virulence during serial passage in cell culture if not serially cloned after isolation. Attenuated mutants of the togaviruses responsible for Japanese B encephalitis [S. Rohitayodhin and W. McD. Hammon, *J. Immunol.* **89**, 823 (1962)] and dengue fever [K. H. Eckels, W. E. Brandt, V. R. Harrison, J. M. McCown, P. K. Russell, *Infect. Immun.* **14**, 1221 (1976)] have also been reported to regain virulence during serial passage in cell culture. Reacquisition of virulence by the attenuated rabies viruses included in our study during serial passage in cell culture (nonneuroblastoma) has never been detected in our laboratory (Table 1) and has not been reported in other laboratories.
3. Poliovirus has been reviewed by A. Jarzabek, W. Sadowski, M. Kantoch, D. Magrath, K. Krawczynski [*Acta. Virol.* **20**, 373 (1976)], by M. Benyesh-Melnick, J. L. Melnick, W. E. Rawls, I. Winberly, J. B. Oro, E. Ben-Porath, V. Rennick [*Am. J. Epidemiol.* **86**, 112 (1967)], by J. M. Hoskin, D. Hobson, V. Udall, T. Madland, A. P. Goffe, C. H. Stuart-Harris, E. G. Herzog [*Br. Med. J.* **1**, 747 (1962)], and by A. Lwoff [*Fifth International Poliomyelitis Conference Copenhagen, Denmark* (1960)]. Influenza virus has been reviewed by J. Mills V and R. M. Chanock [*J. Infect. Dis.* **123**, 145 (1971)]. Fowl plague virus has been reviewed by Y. Z. Ghendon, A. T. Marchenko, S. G. Markushin, D. B. Ghenkina, A. V. Mikheveva, E. E. Rozina, [*Arch. Gesamte Virusforsch.* **42**, 154 (1973)]. Measles virus has been reviewed by D. J. Wear and F. Rapp [*Nature (London)* **227**, 1347 (1970)]. Western encephalitis virus has been reviewed by B. Simizu and N. Takayama [*Arch. Gesamte Virusforsch.* **38**, 328 (1972)].
4. R. J. Klebe and F. H. Ruddle, *J. Cell Biol.* **43**, 69a (1969); J. B. Olmstead, K. Carlson, R. Klebe, F. Ruddle, J. Rosenbaum, *Proc. Natl. Acad. Sci. U.S.A.* **65**, 129 (1970).
5. D. Schubert, S. Humphreys, C. Baroni, M. Cohn, *ibid.* **64**, 316 (1969).
6. F. A. McMorris, A. R. Kolber, B. W. Moore, A. S. Penumal, *J. Cell. Physiol.* **84**, 473 (1974).
7. G. Augusti-Tocco and G. Sato, *Proc. Natl. Acad. Sci. U.S.A.* **64**, 311 (1969).
8. P. Nelson, W. Ruffner, M. Nirenberg, *ibid.*, p. 1004.
9. P. G. Nelson, J. H. Peacock, T. Amano, *J. Cell. Physiol.* **77**, 353 (1971).
10. F. A. McMorris and F. H. Ruddle, *Dev. Biol.* **39**, 226 (1974).
11. A. Komarov and K. Hornstein, *Cornell Vet.* **43**, 344 (1953).
12. H F. Clark and T. J. Wiktor, in *Strains of Human Viruses*, M. Majer and S. A. Plotkin, Eds. (Karger, Basel, 1972), p. 177.
13. H F. Clark and L. Stefanik, unpublished data.
14. H F. Clark and W. Wunner, in preparation.
15. CB-3 is a cow brain cell line established and supplied to us by D. Santoli, Wistar Institute (unpublished).
16. J. J. Tumilowicz, W. W. Nichols, J. J. Cholon, A. E. Greene, *Cancer Res.* **30**, 2110 (1970).
17. J. L. Biedler, L. Helson, B. A. Spingler, *ibid.* **33**, 2643 (1973).
18. Reviewed by Z. Jarzabek, W. Sadowski, M. Kantoch, D. Magrath, K. Krawczynski [*Acta Virol.* **20**, 366 (1976)].
19. Reviewed by A. Lwoff [*Bacteriol. Rev.* **33**, 390 (1969)] and by A. Lwoff and M. Lwoff [*Ann. Inst. Pasteur* **98**, 173 (1960)].
20. H F. Clark and T. J. Wiktor, *J. Infect. Dis.* **125**, 637 (1972); H F. Clark and H. Koprowski, *J. Virol.* **7**, 295 (1971).
21. R. C. Seeger, S. A. Rayner, A. Banijel, H. Chung, W. E. Laug, H. B. Naustein, W. F. Benedict, *Cancer Res.* **37**, 1364 (1977); G. J. West, J. Uki, H. R. Herchman, R. C. Seeger, *ibid.*, p. 1372.
22. Reviewed by X. O. Breakfield [*Life Sci.* **18**, 267 (1976)].
23. S. Matsumoto, *J. Cell Biol.* **19**, 565 (1963).
24. W. D. Sedwick and T. J. Wiktor, *J. Virol.* **1**, 1224 (1967).
25. This work was supported by research grant AI 09706 from the National Institutes of Health. We thank N. Parks for assistance.

7 October 1977; revised 5 December 1977

Cell Walls of Crown-Gall Tumors and Embryonic Plant Tissues Lack *Agrobacterium* Adherence Sites

Abstract. *Crown-gall tumor initiation by* Agrobacterium tumefaciens *is inhibited by cell walls from normal dicotyledonous plants but not by cell walls from crown-gall tumors apparently because of bacterial adherence or nonadherence, respectively, to the different cell walls. Cell walls from normal and tumor tissues in culture also show this difference, indicating that the two types of tissue stably maintain this difference under these conditions. Habituated tissue cultures, which resemble crown-gall tumor cultures, however, form cell walls that are inhibitory like those of the normal cultures from which they are derived. Monocotyledonous plants do not act as hosts for* Agrobacterium *and bacteria-specific inhibition is not shown by cell walls from several species of grass, a monocot family. Cell walls from "embryonic" tissues (dicot seedlings less than 2 centimeters long), unlike those from older seedlings, are noninhibitory. Crown-gall tumors thus resemble embryonic tissues in this respect.*

The transformation of plant cells to a tumorous state by *Agrobacterium tumefaciens* proceeds only after attachment of the bacterium to a site in a plant wound (*1*). Isolated cell walls from susceptible pinto bean leaves were found to exhibit the characteristics expected of the host adherence site (*2*). When these cell walls were inoculated with virulent bacteria on bean leaves, they reduced the number of tumors initiated, apparently by their ability to bind bacteria and thus reduce the number of free bacteria available to attach to actual wound sites where tumor initiation could occur. These cell walls were noninhibitory if added 15 minutes after the bacteria, when bacterial adherence is complete (*1*), or if they were pretreated with an avirulent site-binding strain of bacteria. In examining the effect of cell walls isolated from different plant species, plant parts, and plant tumors, remarkable differences were found in the ability of plant cell walls to inhibit tumor initiation.

Using the procedure of Nevins *et al.* (*3*), we isolated cell walls from 7- to 21-day-old seedlings, "embryonic" tissues from seeds germinated for 3 days (total length < 2 cm), crown-gall tumors in vivo, normal tissues from the same plants, and normal, habituated, and crown-gall tumor tissue cultures. The tissues were blended with an equal volume of 0.5*M* potassium phosphate buffer, *p*H 7.0, and the cell walls were collected by centrifugation for 15 minutes at 2000*g*. They were washed by resuspension and centrifugation three times in this buffer, homogenized with acetone, collected by filtration, and washed three times with fresh acetone. Remaining acetone was removed by overnight evaporation and the dry cell walls were stored in a desiccator.

For infectivity tests, dry cell walls were resuspended in 0.05*M* potassium phosphate buffer, *p*H 7.0, and mixed with an equal volume of washed *A. tumefaciens* strain B6 suspended in the same buffer (*2*). The concentration of cell walls in the samples inoculated was 10 mg (dry weight) per milliliter and the concentration of B6 was 10^8 to 5×10^8 viable bacteria per milliliter. To show that inhibitory effects of cell walls were specific for *Agrobacterium*, a portion of each cell wall preparation was treated with avirulent site-binding strain IIBNV6 of *A. tumefaciens* (5×10^9 cells per milliliter) for 15 minutes. The plant cell walls were then collected by a 10-minute centrifugation at 2000*g*, sus-

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