Table 1. Endogenous tissue levels of cyclic AMP. Values are expressed as the mean ± standard error of the mean of quadruplicate determinations. The number of animals is indicated in parentheses.

Tissue	High cholesterol diet (pmole/μg of DNA)	Control diet (pmole/μg of DNA)
Aorta		
Lesion	0.24 ± 0.03 (6)*	
No lesion	0.09 ± 0.009 (6)	0.09 ± 0.004 (8)
Heart	0.46 ± 0.06 (7)	0.40 ± 0.02 (6)
Liver	0.30 ± 0.03 (12)	0.32 ± 0.04 (10)
Skeletal muscle	0.63 ± 0.07 (11)	0.67 ± 0.10 (7)
Diaphragm	0.99 ± 0.12 (5)	0.85 ± 0.11 (5)

* $P < .001$.

areas without lesions. Those aortas having an insufficient amount of lesion in the designated area were withdrawn from analysis. All tissues were lyophilized and stored at −20°C until used for determination of endogenous cyclic AMP content as described by Steiner and co-workers (*5*). Results obtained from tissues fixed in liquid nitrogen were identical to those obtained with trichloroacetic acid fixation. Cyclic AMP is reported as picomoles per microgram of tissue DNA (*6*).

Plasma cyclic AMP, cholesterol, and triglyceride concentrations from one experiment (eight experimental, eight control animals) are shown in Fig. 1. Cyclic AMP concentrations were not determined for week 7 because of a technical error. There was a significant rise in cyclic AMP in the plasma after week 1 of the cholesterol diet, coincident with the expected increase in plasma cholesterol. The plasma cyclic AMP concentration of cholesterol-fed animals remained significantly elevated ($P < .001$), with some week-to-week variation, throughout the course of the experiment. However, it did not equal the 43-fold overall increase in plasma cholesterol or the 4-fold overall increase in plasma triglycerides. Data from the other experiment supported these conclusions. Such a time-independent but significant increase in the concentration of cyclic AMP is consistent with its role as a "second messenger," that is, a transducer of physiological signals designed to elicit specific biochemical responses without itself effecting the biochemical response (*7*).

The change in the circulating cyclic AMP level demonstrated in the plasma suggested the possibility of a change in the tissue concentration of cyclic AMP. Measurement of endogenous cyclic AMP in several tissues from randomly selected animals from both experiments (Table 1) showed a significantly higher concentration in atherosclerotic lesion areas in aortic intima-media than in adjacent areas where there were no lesions ($P < .001$). Likewise, the cyclic AMP in lesion areas was higher than that in the aortic intima-media of control rabbits. There was no significant elevation of cyclic AMP in heart, liver, skeletal muscle, or diaphragm of the animals on a high cholesterol diet. The specificity of the increase for aortic lesion areas is of special interest in light of the fact that, at 9 weeks, the liver and aorta of animals on the high cholesterol diet had large accumulations of lipid.

The increase in cyclic AMP concentration in plasma and lesion areas is compatible with a disease process characterized by increased lipid permeability and increased cell proliferation. Strange and co-workers (*8*) noted an elevation of plasma cyclic AMP concentration during the first few hours after the onset of symptoms of acute myocardial infarction in man. Bricker and Levey (*9*), using a mammalian liver system, have presented evidence that cyclic AMP may be involved in regulating acetyl–coenzyme A incorporation during de novo fatty acid and cholesterol synthesis. The significance of cyclic AMP concentrations in atherosclerosis has not yet been established. It may be involved in the pathophysiology of the disease, or it may be a secondary consequence. The observation is important because of the regulatory function of cyclic AMP in many cellular processes and because, in plasma, the concentration increase can be demonstrated as early as the increase in plasma cholesterol.

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References and Notes

1. J. P. Jost and H. V. Rickenberg, *Annu. Rev. Biochem.* **40**, 741 (1971).
2. Schwarz/Mann Product Bulletin, *Cyclic AMP Radioimmunoassay Kit* (Schwarz/Mann, Orangeburg, N.Y., 1972).
3. T. C. Huang, C. P. Chen, V. Wefler, A. Raftery, *Anal. Chem.* **33**, 1405 (1961).
4. M. E. Royer and H. Ko, *Anal. Biochem.* **29**, 405 (1969).
5. A. L. Steiner, A. S. Pagliara, L. R. Chase, D. M. Kipnis, *J. Biol. Chem.* **247**, 1114 (1972).
6. G. Ceriotti, *ibid.* **198**, 297 (1952).
7. W. Y. Cheung, *Perspect. Biol. Med.* **15**, 221 (1972).
8. R. C. Strange, N. Vetter, M. J. Rowe, M. F. Oliver, *Eur. J. Clin. Invest.* **4**, 115 (1974).
9. L. A. Bricker and G. S. Levey, *J. Biol. Chem.* **247**, 4914 (1972).
10. We thank E. Collier, J. Einsmann, C. B. Dugan for technical assistance. Supported in part by funds from Veterans Administration research project MRIS 1728, NIH grant HL-14177, and from the New York State Department of Health.

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Measles Virus: An Unwanted Variant Causing Hydrocephalus

Abstract. *Mutagenization of measles virus with proflavine produced a temperature-sensitive mutant capable of inducing hydrocephalus following intracranial inoculation of newborn hamsters. Hydrocephalus was not produced by the parental strain or by other measles virus mutants. Thus, mutants can be the causative agents of disease not associated with the parental strain. The results dictate caution in the use and distribution of experimentally induced virus variants.*

Intracranial inoculation of suckling hamsters with mumps virus not adapted to replication in the brain resulted in the induction of hydrocephalus (*1*). Hydrocephalus, characterized by aqueductal stenosis, was similarly produced with high frequency by intracranial inoculation of influenza virus Ao and parainfluenza virus type 2; but it was not produced by measles virus (*2*). We now report that a mutant strain of measles virus induced hydrocephalus whereas the parental strain did not.

Temperature-sensitive (ts) mutants of measles virus were isolated after the virus had undergone chemical mutagenesis (*3*) and were examined for their neurovirulent potential in the newborn golden Syrian hamster (*4*). During these initial experiments, it was observed that, of eight hamsters autopsied between 18 and 49 days after intracranial inoculation with ts mutant G (tsG), six showed hydrocephalus char-

acterized by extreme dilation of the lateral ventricles. An additional two animals killed after 8 months showed similar pathology. This mutant had been derived after treatment of the parental measles virus with proflavine (7.5 μg/ml). To determine whether tsG could reproducibly induce hydrocephalus with high frequency, we inoculated newborn hamsters with tsG previously treated with either specific antiserum to measles virus or with nonimmune serum (*5*). Of 55 animals examined between 14 and 33 days after inoculation with nonneutralized virus, 42 (76 percent) were hydrocephalic (Table 1). Hydrocephalus occurred in all litters, ranging in frequency from 50 to 100 percent of the hamsters. There were degrees of hydrocephalus ranging from moderate to severe dilation of the lateral and third ventricles with disruption of normal brain structure (Fig. 1). Mutant G was recovered from the brains of two hydrocephalic animals tested for the presence of the virus 14 and 18 days after inoculation. Examination of the 18 hamsters inoculated with "neutralized" virus revealed that only one animal developed a moderate case of hydrocephalus; the pathogenesis of hydrocephalus was therefore inhibited by specific antibody to measles virus ($P > .001$; Fisher's exact two-by-two test). The stenosis of the midportion of the aqueduct was characterized by a loss of ependymal cells and the presence of inflammatory cells in the ependymal cell layer as well as within the aqueduct. Extensive resolution of inflammation was observed 1 month after inoculation. The inflammatory process associated with hydrocephalus was similarly prevented by prior treatment with immune serum.

Pressure of the cerebrospinal fluid often resulted in distension of the brain with a caudal-ventral displacement of the cerebellum. Many of the hydrocephalic animals had deformed dome-shaped skulls. Although some animals exhibited ataxia, most hydrocephalic animals were asymptomatic even as long as 8 months after inoculation.

The ability of mumps virus to induce hydrocephalus was not associated with a specific strain, but was due rather to an inability to produce acute encephalitis. Attenuation of neurovirulence does not account for the ability of tsG to induce hydrocephalus, as this encephalopathy did not evolve as a sequel to intracranial inoculation of five other similarly attenuated ts mutants of measles virus (Table 1). It is obvious that some property unique to tsG is responsible for its unusual ability to produce hydrocephalus. The pathogenetic mechanism of this unexpected neurological disorder remains to be determined. It has been suggested that a mutant strain of measles virus may be involved in the pathogenesis of subacute sclerosing panencephalitis (*6*). Our observation that tsG, unlike other strains of measles virus, produced hydrocephalus is in agreement with the reported alteration of neuropathogenic properties of ts mutants of reovirus (*7*). These findings support the concept that mutants can indeed induce aberrant disorders not observed during infection with the wild-type virus.

Table 1. Incidence of hydrocephalus after intracranial inoculation of measles virus. Hamsters were inoculated with 3×10^4 plaque-forming units within 24 hours of birth.

Inoculum	Animals autopsied (No.)	Hydrocephalus (No.)	Percent
Noninfected cell homogenate	22	0	0
Parental virus	6	0	0
Five other ts mutants (combined data)	43	0	0
Ts mutant G (untreated)	11	8	73
Ts mutant G (prior treatment with nonimmune serum)	55	42	76
Ts mutant G (prior treatment with immune serum)	18	1	5

Recently, there has been increasing controversy concerning the advisability of conducting genetic experiments involving the production of new autonomously replicating bacterial plasmids, hybrids of animal virus and plasmid DNA molecules, and recombinant influenza viruses (*8*). It is also possible to select for measles virus variants that are resistant to neutralization (*9*). Our studies demonstrate that in isolating ts mutants, the "workhorse" of animal virus genetics, one may inadvertently produce mutants capable of inducing diseases not associated with the parental strain. Therefore, it is our opinion that virus mutants should be considered potentially more hazardous than the parental strain and should be handled and treated accordingly.

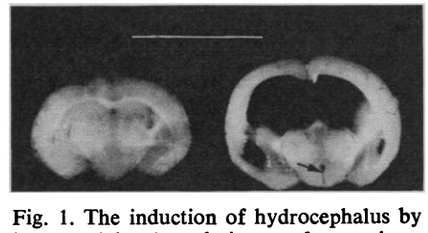

Fig. 1. The induction of hydrocephalus by intracranial inoculation of newborn golden Syrian hamsters with 3×10^4 plaque-forming units of ts mutant G of measles virus. The coronal section on the left is from an animal that was inoculated 33 days earlier with mutant G that had been treated with measles virus-specific antiserum. Hydrocephalus is absent. The coronal section on the right is from an animal that was inoculated 33 days earlier with mutant G that had been first treated with nonimmune serum. The severe hydrocephalus results in disruption of normal brain architecture. The abnormally dilated third ventricle (arrow) can be observed at the ventral aspect of the brain.

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References and Notes

1. R. T. Johnson, K. P. Johnson, C. J. Edmonds, *Science* **157**, 1066 (1967); R. T. Johnson and K. P. Johnson, *J. Neuropathol. Exp. Neurol.* **27**, 591 (1968).
2. R. T. Johnson and K. P. Johnson, *Exp. Mol. Pathol.* **10**, 68 (1969).
3. M. V. Haspel, R. Duff, F. Rapp, in preparation.
4. ———, in preparation. Newborn hamsters were inoculated by the intracranial route with 3×10^4 plaque-forming units in 0.05 ml.
5. TsG at a final concentration of 6×10^5 plaque-forming units per milliliter was incubated at room temperature for 1 hour with either nonimmune serum or with rabbit hyperimmune serum prepared against purified measles virus (obtained from Dr. E. Norrby, Karolinska Institute). Golden Syrian hamsters were inoculated with 0.05 ml by the intracranial route within 24 hours of birth. Additional animals were inoculated with a clarified homogenate of noninfected BSC-1 cells.
6. R. T. Johnson, *J. Infect. Dis.* **121**, 227 (1970).
7. B. N. Fields, *N. Engl. J. Med.* **287**, 1026 (1972).
8. P. Berg, D. Baltimore, H. W. Boyer, S. N. Cohen, R. W. Davis, D. S. Hogness, D. Nathans, R. Roblin, J. D. Watson, S. Weissman, N. D. Zinder, *Science* **185**, 303 (1974); M. Stoker, *Nature (Lond.)* **250**, 278 (1974); E. S. Anderson, *ibid.*, p. 279; K. Murray, *ibid.*, p. 279; E. D. Kilbourne, *Science* **184**, 414 (1974).
9. F. E. Payne and J. V. Baublis, *J. Infect. Dis.* **127**, 505 (1973).
10. We thank Drs. Ronald Duff of Abbott Laboratories, North Chicago, Illinois; and Robert W. Brennan, John W. Kreider, and Bryce L. Munger of the Milton S. Hershey Medical Center for their interest and assistance. Supported in part by NCI contract 70-2024 within the Virus Cancer Program and PHS research grant 1R01 AI12203. M.V.H. is a recipient of NIH research fellowship award 1F22 NS00459.

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