mouse embryo, and we related this interference to the pathogenesis of cleft lip and palate (*14*). In addition, this treatment retards the schedule of embryonic development by 4 to 6 hours (*15*) and produces hypoplasia of the lateral nasal prominences (*16*). These manifestations of abnormal development may result from interference with critical interactions between mother and embryo. Since hyperoxia is so effective in reducing the teratogenicity of phenytoin, it is tempting to speculate that the drug, and its alkaline vehicle in this case, by depressing maternal cardiorespiratory function, indirectly affects embryonic development. This maternally mediated mechanism of teratogenesis can be partly compensated for by increasing the O_2 tension, which restores oxygen delivery to the mother and embryo. The results of the present study are supported by evidence reported by Millicovsky and his co-workers (*17*) that injection of substances that acutely alter maternal cardiovascular function can severely affect the structure and function of the embryonic cardiovascular system and produce craniofacial, limb, and trunk defects. It may be that phenytoin injection decreases uterine circulation, since an earlier study has shown that brief reductions in uterine blood flow at critical times in pregnancy may disrupt embryonic cardiac function and severely compromise the craniofacial region of the developing embryo (*18*). This finding substantiates our hypothesis that the depression of cardiorespiratory function elicited by phenytoin in A/J mice may be responsible in part for the facial clefts seen in the term fetuses. The results of our study demonstrate that a drug does not have to reach the embryo to produce malformations. This concept has general implications for the possible roles of maternal toxicity on teratogenesis.

Although we have shown here that respiratory hyperoxia (50 percent O_2 and 50 percent N_2) reduces the teratogenic effect of phenytoin, it is necessary to determine if elevated O_2 concentrations help the embryo by compensating for depression in maternal cardiorespiratory function or by reducing other effects of phenytoin (*3*) in the maternal-placental-embryonic complex (*19*).

GUILLERMO MILLICOVSKY
Dental Research Center, University of North Carolina, Chapel Hill 27514
MALCOLM C. JOHNSTON
Dental Research Center and *Department of Orthodontics, School of Dentistry*, and *Department of Anatomy, School of Medicine, University of North Carolina*

References and Notes

1. H. H. Merritt and T. J. Putnam, *J. Am. Med. Assoc.* **111**, 1068 (1938).
2. S. R. Meadow, *Lancet* **1968-II**, 1296 (1968).
3. D. W. Nebert, in *Phenytoin-Induced Teratology and Gingival Pathology*, T. M. Hassell, M. C. Johnston, K. H. Dudley, Eds. (Raven, New York, 1980), pp. 113–128.
4. P. G. Wells and D. Harbison, in *ibid.*, pp. 83–111.
5. K. M. Massey, *J. Oral Ther. Pharmacol.* **2**, 380 (1966); J. E. Gibson and B. A. Becker, *Proc. Soc. Exp. Biol. Med.* **128**, 905 (1968).
6. M. C. Johnston, K. K. Sulik, K. H. Dudley, *J. Dent. Res.* **57A**, 326 (1978). The cleft palate associated with cleft lip is thought to result from the cleft lip [D. G. Trasler and F. C. Fraser, *Dev. Biol.* **6**, 45 (1963); G. R. Smiley, R. J. Vanek, A. D. Dixon, *Cleft Palate J.* **8**, 371 (1971)].
7. As defined by T. W. Rall and L. S. Schleifer, in *The Pharmacological Basis of Therapeutics*, A. G. Gilman, L. S. Goodman, A. Gilman, Eds. (Macmillan, New York, 1980), pp. 454–455.
8. G. Millicovsky and W. P. Watkinson, *Teratology*, in press.
9. These unpublished observations were made on A/J mice injected with phenytoin (40, 60, or 75 mg/kg) in our laboratory.
10. J. B. West, in *Respiratory Physiology—The Essentials* (Williams and Wilkins, Baltimore, 1974), pp. 51–71.
10a. C. Petter, J. Bourbon, J. P. Maltier, A. Jost, *C.R. Acad. Sci. Ser. D* **273**, 2639 (1971).
11. This precise timing of the injection represents the peak of phenytoin sensitivity in this drug-animal model for the production of cleft lip and palate (*6*).
12. F. C. Fraser, *Am. J. Hum. Genet.* **22**, 336 (1970); in *Handbook of Teratology*, J. G. Wilson and F. C. Fraser, Eds. (Plenum, New York, 1978), pp. 75–96.
13. G. Millicovsky and M. C. Johnston, unpublished results.
14. ———, *Teratology* **21**, 56A (1980); *J. Embryol. Exp. Morphol.*, in press.
15. K. K. Sulik and M. C. Johnston, unpublished data.
16. ———, L. J. H. Ambrose, D. R. Dorgan, *Anat. Rec.* **195**, (1979).
17. G. Millicovsky and J. M. DeSesso, *Teratology* **17**, 18A (1978); G. Millicovsky, K. E. Clark, J. M. DeSesso, *ibid.* **19**, 39A (1979); G. Millicovsky and J. M. DeSesso, *ibid.* **22**, 115 (1980).
18. G. Millicovsky, L. J. Millicovsky, J. M. DeSesso, *ibid.* **19**, 39A (1979); G. Millicovsky and J. M. DeSesso, *ibid.* **22**, 335 (1980).
19. Future studies will be aimed at maximizing the antiteratogenicity of hyperoxia by varying the O_2/N_2 ratio, humidity, temperature, and the length of time that the animals are kept in the environmental chambers.
20. E. L. Lehmann, *Nonparametrics: Statistical Methods Based on Ranks* (Holden-Day, San Francisco, 1975).
21. J. K. Haseman and D. G. Hoel, *J. Stat. Comput. Simulation* **3**, 117 (1974).
22. We thank P. Hirsch, R. Mueller, J. Karon, and J. Goulson for their comments and R. Williams for typing the manuscript. Supported by National Institutes of Health fellowship DE 05248 and grants DE 02668 and RR 05333.

6 November 1980

Measles Virus Nucleotide Sequences: Detection by Hybridization in situ

Abstract. *A tritium-labeled probe that detects measles virus nucleotide sequences was hybridized in situ to cells infected with measles virus and to sections of brain tissue from patients with subacute sclerosing panencephalitis and from patients with multiple sclerosis. The measles virus genome was detected in many cells in subacute sclerosing panencephalitis where this virus would have been missed by methods such as immunofluorescence. Measles virus sequences were also found in two foci in one of four cases of multiple sclerosis. This refined method of hybridization in situ, which can be useful in the search for covert virus infections of man, provides evidence that viruses may be involved in multiple sclerosis.*

In a number of diseases of animals caused by viruses or virus-like agents, symptoms appear long after the infectious process is initiated (*1*). The relevance of these slow infections to diseases of humans was recognized when diseases like kuru and Creutzfeldt-Jakob disease were shown to be transmissible (*2*). Subsequently, the consistent isolation of viruses and the demonstration of viral antigens and particles in affected organs linked a measles-like virus and a papovavirus, respectively, to subacute sclerosing panencephalitis (SSPE) and progressive multifocal leukoencephalopathy (PML) (*3*, *4*). There are considerable indirect and epidemiological data suggesting that more prevalent human illnesses, including multiple sclerosis (MS), may also be slow infections, with measles virus as the most likely infective agent (*5*), but reproducible evidence of the kind just cited has not been found.

If the measles virus genome is present in MS, but focally distributed and restricted in genetic expression, as is the case in visna, a slow infection of sheep (*6*, *7*), then infectious virus, antigens, or particles would not be detected, and viral genetic sequences might not be discovered by conventional hybridization methods. The visna provirus was identified in infected tissues by hybridization in situ (*7*); as now refined, this method is capable of detecting less than a single copy of viral sequences in individual cells (*8*). We therefore used this method to assay brain tissues of patients with MS and with SSPE, the latter serving as a positive control. We can detect the measles virus genome in cells in tissue sections from cases of SSPE in which viral antigens were undetectable by immunofluorescence. We have found the measles virus genome in one of four cases of MS.

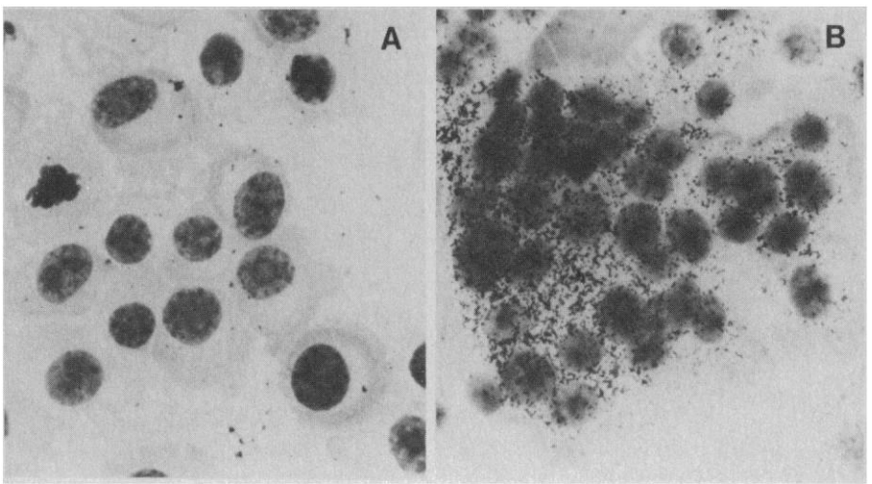
The virus-specific tritium-labeled probe used in these studies was prepared by reverse transcription of full-length measles RNA purified from a heat-resis-

0036-8075/81/0508-0672$00.50/0

tant Edmonston strain of measles virus designated HRE (*9*). The probe had specific activity of 1.9×10^8 dpm/μg and was hybridized to cells and tissues as described in (*8*), with minor modifications (*10*). Under these conditions, uninfected Vero cells had background levels of hybridization of two grains per cell with 3 weeks of radioautographic exposure (Fig. 1). Hybridization specifically detects measles virus RNA because digestion with ribonuclease (*7*) before hybridization reduced grain counts to the level of uninfected cells, and only background levels of hybridization were observed with a probe specific for visna virus.

In sections of tissues obtained by brain biopsy from cases of SSPE in which viral antigens were not detectable by immunofluorescence (*11*), small amounts of measles virus nucleotide sequences were detectable by hybridization in situ (Table 1). Viral sequences were found in individual cells (Fig. 2, A, B, and C) and in the neuropil (Fig. 2, B and C). The pattern of the latter reflects accumulation of viral RNA in dendritic processes, since a similar pattern is seen in hamsters infected with measles virus (*12*).

In one of four cases of MS there were two foci with corresponding patterns of grains over neurons and glial cells (Fig. 2C) and in the neuropil (Fig. 2, D and E). One focus was adjacent to a demyelinated plaque (Fig. 2, C and D); the other focus was in a relatively unaffected area (Fig. 2E). Identical results were obtained in two subjacent sections hybridized independently at other times. The low background level of hybridization (in an area adjacent to that shown in Fig. 2D) is evident in Fig. 2F. Comparable background levels of hybridization were observed in sections from five other relatively unaffected areas in this patient, from sections with plaques in this patient and other patients with MS, and in the tissues from patients dying of unrelated causes. The specificity of the hybridization result in the positive foci meets the



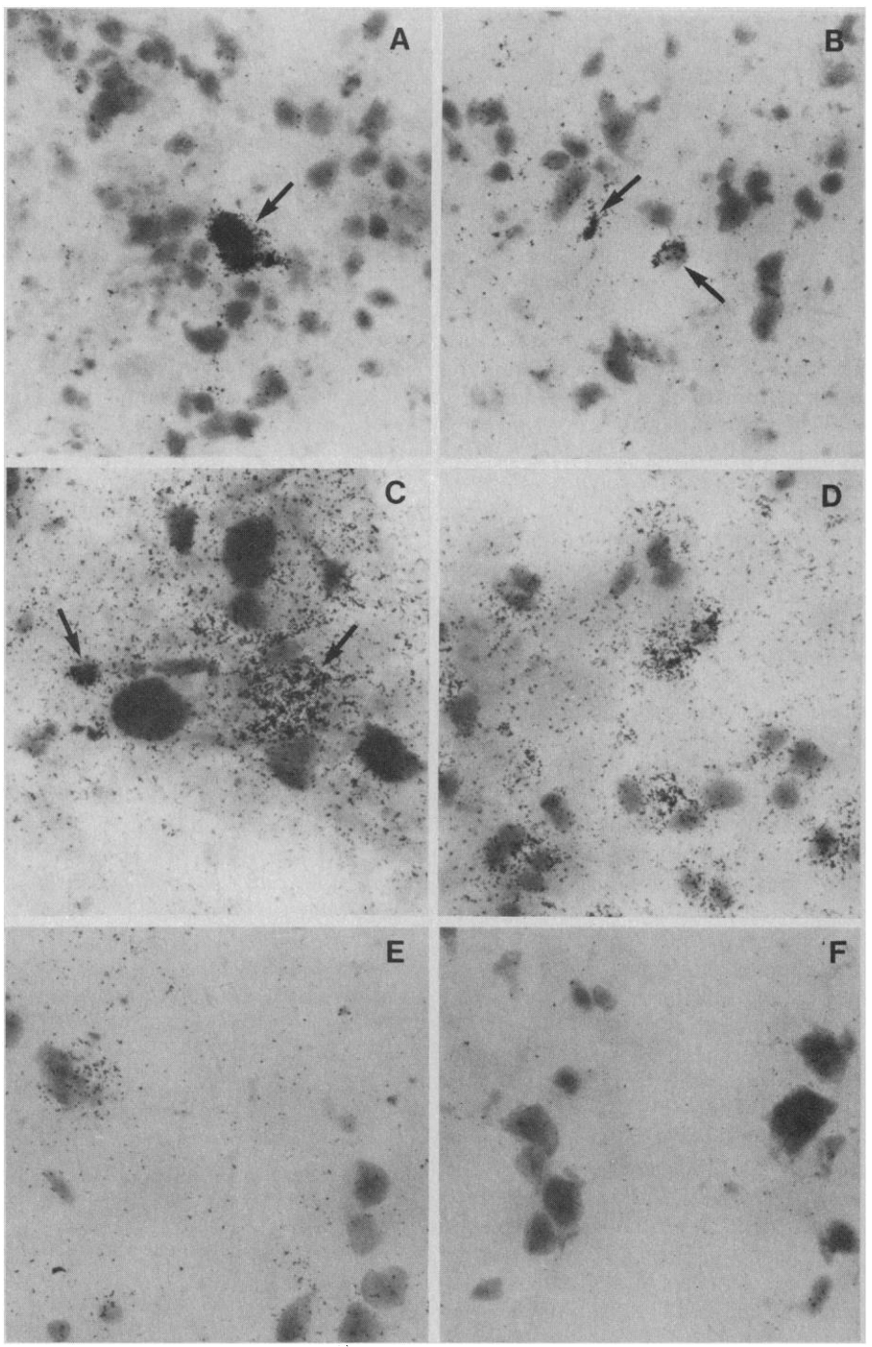


Fig. 1 (top). Demonstration of measles virus RNA in tissue culture cells. Cells were hybridized to tritium-labeled DNA as described in the text. (A) Uninfected control; radioautographic exposure, 3 weeks. (B) Infected cell; radioautographic exposure, 4 hours. Original magnification, ×600. Fig. 2 (bottom). Measles virus RNA in (A and B) subacute sclerosing panencephalitis and (C-F) multiple sclerosis. Tissue sections were hybridized to tritium-labeled measles complementary DNA and developed after 3 weeks. Arrows in (A), (B), and (C) point to grains over cells and to grains in the neuropil. Original magnification, ×600.

Table 1. Source of tissues hybridized in situ. The diagnosis of multiple sclerosis (MS) was based on the characteristic clinical features, course (remissions and exacerbations), elevated protein (immunoglobulin G) in the spinal fluid, and pathology (primary demyelination). The cases of SSPE are described in (*14*).

Case	Diagnosis	Age	Sex	Autolysis time (hours)	History of measles	Treatment	Hybridization result
1	MS	51	F	3	+	ACTH	+
2	MS	42	M	6	+	ACTH	−
3	MS	56	M	23	+	ACTH	−
4	MS	44	F	10	+	ACTH	−
5	SSPE		M	4	+	Unknown	+
6	SSPE	11	M	2	+	Unknown	+
7	SSPE	4	F	2	+	Unknown	+
8	SSPE	18	F	6	+	Unknown	+
9	Blunt force trauma to face and neck	19	M	7	Unknown		−
10	Alzheimer's disease	67	M	24	Unknown		−
11	Heart attack	33	M	16	Unknown		−
12	Huntington's disease	68	F	12	Unknown		−

criteria defined for Vero cells; only background levels of hybridization were observed in foci in subjacent sections that were digested prior to hybridization with ribonuclease or in sections hybridized to a probe for visna virus sequences of comparable specific activity.

These studies demonstrate the usefulness of hybridization in situ in locating virus-specific sequences in tissues. We estimate from grain counts and length of exposure (*13*) that with rare exceptions, such as the cell shown in Fig. 2A, the positive cells in SSPE contain fewer than ten copies of the measles virus genome per cell; the positive cells from the patient with MS contain about one copy per cell. Viral infection of this kind would not be recognized by application of conventional hybridization methods in which nucleic acid extracted from tissues is annealed in solution to a virus-specific probe, because of the minute quantities of virus-specific nucleotide sequences (10^{-17} g per cell) in the occasional cell and because of the focal nature of the infection. Methods such as immunofluorescence that do assess infection at the level of the single cell are not sensitive enough to detect infection in which there is a low copy number, presumably because insufficient antigen is synthesized.

The experiments provide evidence for a subtle association between MS and the genome of measles virus, or one closely related to it, but the implications for the etiology and pathogenesis of the disease are not clear. If we require—as a restatement of Koch's postulates in molecular equivalents—a constant association of the viral genome with a disease in a plausible site to cause that disease, neither condition is satisfied for MS. It is possible that the measles virus genome is present in a much higher proportion of cells, but was not detected because of the limited number of tissue sections we sampled, or because of loss of viral RNA with longer autolysis times. The fact that the virus genome was found in both pathologically involved and uninvolved areas, and in neurons as well as oligodendrocytes, suggests that the virus induces primary demyelination by an indirect mechanism, perhaps by provoking tissue damage as a consequence of the host's immune and inflammatory response. Alternatively, measles virus may be an adventitious agent in this setting of a debilitated host undergoing treatment with steroids.

A. T. HAASE
P. VENTURA
Veterans Administration Medical Center and *University of California, San Francisco 94121*
C. J. GIBBS, JR.
National Institutes of Health, Bethesda, Maryland 20205
W. W. TOURTELLOTTE
Neurology and Research Services, Veterans Administration, Wadsworth Medical Center, Los Angeles, California 90073, and *Department of Neurology, University of California, Los Angeles 90073*

References and Notes

1. B. Sigurdsson, *Br. Vet. J.* **110**, 341 (1954).
2. D. C. Gajdusek, *J. Clin. Pathol.* **25** (Suppl.), 78 (1972).
3. J. V. Baublis and F. E. Payne, *Proc. Soc. Exp. Biol. Med.* **129**, 593 (1968); M. Bouteille, C. Fontaine, C. Vedrenne, J. Delarue, *Rev. Neurol.* **113**, 454 (1965); T. T. Chen, I. Watanabe, W. Zeman, J. Mealey, Jr., *Science* **163**, 1193 (1969); J. H. Connolly, I. V. Allen, L. J. Jurwitz, J. H. D. Miller, *Lancet* **1967-I**, 542 (1967); A. D. Dayan, J. V. T. Gostling, J. L. Greaves, D. W. Stevens, M. A. Woodhouse, *ibid.*, p. 980; J. M. Freeman, R. L. Magoffin, E. H. Lennette, R. M. Herndon, *ibid.* **2**, 129 (1967); L. Horta-Barbosa, J. Sever, W. Zeman, *Nature (London)* **221**, 974 (1969); N. J. Legg, *Br. Med. J.* **3**, 350 (1967); I. Tellez-Nagel and D. H. Harter, *Science* **154**, 899 (1966).
4. O. Narayan, J. B. Penney, Jr., R. T. Johnson, R. M. Herndon, L. P. Weiner, *N. Engl. J. Med.* **289**, 1278 (1973); B. L. Padgett, D. L. Walker, G. M. ZuRhein, R. J. Eckroode, B. H. Dessel, *Lancet* **1971-I**, 1257 (1971); L. P. Weiner *et al.*, *N. Engl. J. Med.* **286**, 385 (1972); J. B. Penney, Jr., L. P. Weiner, R. M. Herndon, O. Narayan, R. T. Johnson, *Science* **178**, 60 (1972); G. M. ZuRhein, *Prog. Med. Virol.* **11**, 185 (1969); E. P. Richardson, Jr., *N. Engl. J. Med.* **265**, 815 (1961).
5. J. Prineas, *Science* **178**, 760 (1972); D. C. Poskanzer, K. Shapira, H. Miller, *Lancet* **1973-II**, 917 (1973); J. H. D. Miller, *Multiple Sclerosis* (Thomas, Springfield, Ill., 1971), p. 12; M. Alter, in *Progress in Multiple Sclerosis*, U. Liebowitz, Ed. (Academic Press, New York, 1972); J. M. Adams and D. T. Imagawa, *Proc. Soc. Exp. Biol. Med.* **111**, 562 (1962); E. Norrby, *Prog. Med. Virol.*, in press; K. B. Fraser, *Br. Med. Bull.* **33**, 34 (1977); L. P. Weiner, R. T. Johnson, R. M. Herndon, *N. Engl. J. Med.* **288**, 1103 (1973).
6. A. T. Haase, *Curr. Top. Microbiol. Immunol.* **72**, 101 (1975).
7. A. T. Haase, L. Stowring, O. Narayan, D. Griffin, D. Price, *Science* **195**, 175 (1977).
8. M. Brahic and A. T. Haase, *Proc. Natl. Acad. Sci. U.S.A.* **75**, 6125 (1978).
9. Measles virus RNA was purified from Vero cells infected at low multiplicity with a strain of Edmonston virus selected for growth at 39°C. Viral nucleocapsids in the cytoplasmic fraction were separated from host ribonucleoprotein in Renografin step gradient [L. A. Caliguiri and H. Gerstein, *Virology* **90**, 119 (1978)] and deproteinized and sedimented in a sucrose density gradient [W. W. Hall and V. ter Meulen, *Nature (London)* **264**, 474 (1976)] to obtain 50*S* viral RNA free from residual host RNA. Tritium-labeled DNA was prepared by reverse transcription of the viral RNA with avian myeloblastosis virus polymerase and random primers generated by digestion of calf thymus DNA [J. M. Taylor, R. Illmensee, J. Summers, *Biochim. Biophys. Acta* **442**, 324 (1976)]. The tritium-labeled probe was subsequently deproteinized, treated with alkali to reduce the size of the probe and to digest viral RNA, neutralized, and chromatographed on Sephadex G-50 to separate the tritium-labeled complementary DNA (cDNA) from unincorporated precursors and primers.
10. For hybridization in situ, 10-μm sections of frozen tissues were cut with a cryostat (*7*) and picked up on slides that had been coated with Denhardt's hybridization medium and subsequently acetylated [(*8*); S. Hayashi, I. C. Gillam, A. D. Delaney, G. M. Tener, *J. Histochem. Cytochem.* **26**, 677 (1978)]; cells were deposited on slides with a cytocentrifuge. Before hybridization, the cells or tissues were fixed in a mixture of ethanol and acetic acid, treated with HCl heated to 70°C, and digested with proteinase K to increase diffusion of the probe. Measles cDNA, 2 ng per slide, was precipitated in ethanol with 25 μg of RNA from uninfected Vero cells as carrier, centrifuged, dried in vacuo, and brought up in 5-μl of hybridization medium per slide, which contained 50 percent (by volume) deionized formamide, 10 percent (weight to volume) dextran sulfate, 0.6*M* NaCl, 10 mm tris-HCl (*p*H 7.4), 0.02 percent (weight to volume) each of acetylated bovine serum albumin, polyvinylpyrrolidine, and Ficoll, polyadenylate (100 μg/ml) and RNA from uninfected Vero cells (1 mg/ml). Hybridization was carried out under siliconized cover slips under oil at 24° to 26°C; subsequent steps of washing and radioautographic procedures followed established protocols (*7, 8*).
11. Sections subjacent to those used for hybridization in situ were fixed in acetone and assayed for viral antigens by indirect immunofluorescence with a high-titered antiserum to measles virus from a patient with SSPE, and a fluorescein-conjugated rabbit antiserum to human immunoglobulin G.
12. A. T. Haase, in preparation.
13. With a specific activity of about 2×10^8 dpm/μg, hybridization in situ at 100 percent efficiency, and 0.1 grain per dpm, one copy of measles RNA per cell with a molecular weight of 6×10^6 would give approximately 20 grains per cell with a 3-week exposure. With dextran sulfate [G. M. Wahl, M. Stern, G. R. Stark, *Proc. Natl. Acad. Sci. U.S.A.* **76**, 3683 (1979)], and a partially duplex DNA, networks of DNA form that increase the radioactivity per site about

threefold (A. T. Haase, unpublished data). Most positive cells contained 50 to 100 grains, or one to two copies per cell.

14. W. Zeman, in *Handbook of Clinical Neurology. Infections of the Nervous System*, part 2, P. J. Vinken and G. W. Bruyn, Eds. (North-Holland, New York, 1978), vol. 34, chap. 18, pp. 343–368.

15. Supported by the Veterans Administration and by grants from the National Institutes of Health, National Multiple Sclerosis Society, the American Cancer Society, and the Herechray Disease Foundation. We thank P. Swoveland for reagents used in immunofluorescence assay, M. Brahic and J. Harris for helpful comments on the manuscript, and H. Lukes for its preparation.

9 January 1981

Oxalate Degradation by Microbes of the Large Bowel of Herbivores: The Effect of Dietary Oxalate

Abstract. *Rates of oxalate degradation by microbes in gastrointestinal contents from rabbits, guinea pigs, swine, and a horse increased after addition of oxalate to diets. A similar response was previously observed with ruminal microbes from cattle and sheep. Bacteria that utilize oxalate for growth appear to be selected by increased levels of dietary oxalate.*

Increased rates of oxalate degradation by ruminal microbes account for ruminant adaptation to high levels of dietary oxalate (*1*). Adapted ruminants tolerate quantities of oxalate that would be lethal to nonadapted animals (*2*). The experiments reported here show that high levels of dietary oxalate also induce increases in rates of oxalate degradation by microbes in the large bowel of various nonruminant herbivores.

Low levels of oxalic acid are found in many plants that are important in the diets of man and other animals (*3*). Problems due to ingested oxalate arise mainly when: (i) foods containing much higher concentrations of oxalate abruptly become major components of the diet, or (ii) when gastrointestinal function is altered and a significantly increased proportion of the dietary oxalate is absorbed. Nephrolithiasis resulting from increased oxalate absorption is a frequent and serious complication of inflammatory small bowel disease or resection of the terminal ileum (*4*).

Degradation of oxalate by gastrointestinal microbes from humans (*5*), swine (*6*), and certain rodents (*7*) has been shown, but degradation rates have not been measured and specific organisms responsible for this activity have not been identified. Although oxalate degradation by aerobic bacteria has been extensively studied (*3*), these organisms are unlikely to be important in an anaerobic ecosystem such as the rumen or large bowel. An anaerobic bacterium that degrades oxalate to CO_2 and formate was, however, recently isolated from rumen contents (*8*). This obligate anaerobe (now called strain OxB because it has not yet been placed taxonomically) is apparently unable to use any substrate other than oxalate for growth. This property could explain how increased amounts of dietary oxalate select for this organism and result in ruminal populations that degrade oxalate at greatly increased rates.

Laboratory animals were given free access to gradually increasing amounts of oxalate, the normal laboratory diet being replaced with ground *Halogeton glomeratus* (halogeton) plant material. The halogeton contained 14.7 percent oxalate, most of which was present as the soluble sodium salt (*3*). After 2 days with 5 percent of the diet as halogeton and 2 days with 10 percent halogeton, the halogeton was fed at a 20 percent level so that oxalate constituted 2.9 percent of the diet. The animals were killed 7 days after the start of halogeton feeding. Oxalate degradation rates were then estimated as described (*1*) by measurement of $^{14}CO_2$ produced in vitro during short-term incubation of the gut contents with ^{14}C-labeled oxalate.

Mean rates of oxalate degradation by microbes in cecal contents from rabbits and guinea pigs fed diets that contained halogeton were significantly ($P \leq .001$) greater than rates with cecal contents from animals fed the normal diets (Table 1). With cecal contents from white laboratory rats, however, oxalate degradation rates were very low in samples from animals fed either halogeton or the control diet, and conclusive evidence for selection of oxalate-degrading microbes in the rat cecum was not obtained.

When a pig (48 kg) with a surgically implanted cecal cannula was fed a diet containing 10 percent and later 20 percent halogeton, the rates of oxalate deg-

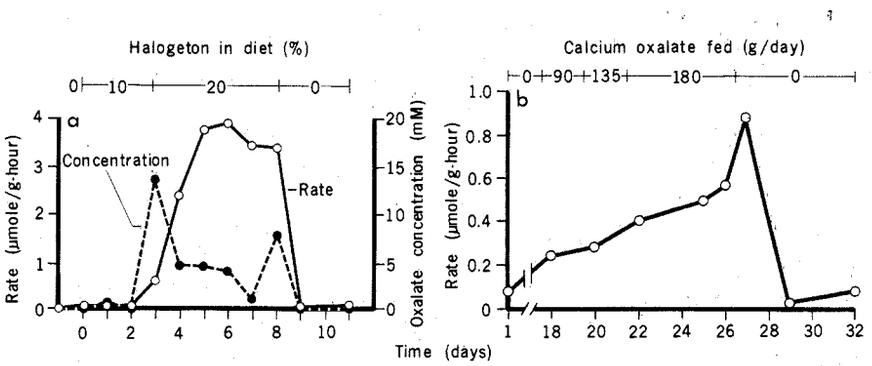


Fig. 1. Rates of oxalate degradation (solid lines) by microbes in (a) cecal contents from a pig and (b) rectal contents from a horse. Degradation rates were measured as in Table 1; except with pig cecal contents, ^{14}C-labeled oxalate was incubated with a filtrate that passed through muslin cloth. Soluble oxalate, measured by gas chromatography of the dibutyl ester (*1*), was not detected in rectal contents from the horse.

Table 1. Mean rates of oxalate degradation by microbes in cecal contents from laboratory animals. Tests for rabbits and guinea pigs were with cecal contents from individual animals (three on each diet), whereas with rats, cecal contents from two animals were pooled for each test (six animals on each diet). The reaction mixtures, which were incubated (in duplicate) under CO_2 for 1 hour at 37°C, consisted of 1.8 ml of a 1:4 dilution of cecal contents in an anaerobic dilution solution (*10*) plus 0.2 ml of 0.1*M* ^{14}C-oxalate (0.02 μCi/μmole). One milliliter of 1*N* NaOH was injected through the rubber stopper to stop each reaction. $^{14}CO_3^{2-}$ was measured by liquid scintillation counting after diffusion (*1*) into Carbo-sorb (Packard Instrument Co.). Controls included tubes stopped at 0 minute. Student's *t*-test was used to determine confidence intervals (logarithmic transformation) on the ratios of rates of oxalate degradation in halogeton diet to those in control diets.

Animal	Rate of oxalate degradation		95 percent confidence interval on ratio halogeton/control
	Control diet (μmole/g-hour)	Halogeton diet (μmole/g-hour)	
Rabbits	0.48	5.8	7.4 to 20.1
Guinea pigs	0.68	7.2	4.6 to 24.4
Rats	0.02	0.08	0.45 to 250

0036-8075/81/0508-0675$00.50/0