and the angle of minimum deviation is 27.46° if the refractive index of ice is taken as 1.307. Octahedral crystals would therefore cause only one halo, due to refraction through the octahedral faces o_1o_3, o_1o_6, o_1o_8, . . . , as labeled in Fig. 1, and it would be at the angle reported for Scheiner's halo. Scheiner's halo could therefore be caused by octahedral crystals of cubic ice.

Truncated octahedral crystals, as illustrated in Fig. 1, could form another halo due to refraction through one cubic and one octahedral face, such as a_1o_5. The prism angle is 54.736°, and it would produce a halo at 19.12°. This is very close to Burney's halo at 19.0° (*17*), which is usually attributed to refraction through the faces (01.1) and $(0\bar{1}.1)$ (p_1p_4), which should give a halo at 19.7° if the axial ratio *c*/*a* is 1.624 (*20*). The halo at 27.46° is therefore the only halo that is firmly characteristic of ice Ic crystals having low-index faces.

If a droplet of water in the upper atmosphere nucleates to form ice Ic, it will heat to the melting point in times of the magnitude of some microseconds (*12*) and will cool in times of the magnitude of some milliseconds. There is evidence from two sources that, in spite of this, single crystals of ice Ic will survive long enough for a halo to be observed. The first is an experiment in which a bulk sample of ice Ic was heated (*10*) to successive temperatures, each 10 K higher than the preceding. It was kept at each temperature for 4 minutes, cooled to 90 K, and x-ray powder photographs taken. After 208 K no Ih was present, after 218 K it was noticeable, and after 228 K the ice Ic had completely transformed to Ih. In this bulk sample, each particle that transformed could help nucleate its neighbors. If small octahedral crystals are produced in the atmosphere, they probably have a much longer life than this sample, partly because they are isolated from one another and partly because they may be only about 10 μm across, and so nucleate slowly.

The second is an experiment in which Oguro and Higashi found stacking faults by x-ray topography in ammonia-doped (*21, 22*) and pure (*23*) ice. Each fault is a thin layer of ice Ic sandwiched between ice Ih; it may be several millimeters across, and so similar in volume to a 10-μm octahedron. The faults survive for hours or days near 0°C, which suggests that a small crystal of ice Ic may survive heating to 0°C for 1 msec or so.

It therefore appears that Scheiner's halo is probably caused by ice Ic. If this is true, it shows that (i) liquid water can freeze to ice Ic at $\sim$ 230 K and (ii) ice Ic sometimes occurs in the upper atmosphere. These points suggest that when emulsions of water are frozen at $\sim$ 183 K at a pressure of 2 kbar (*24*) they probably form some ice Ic and that ice Ic may have some meteorological significance. It may be possible to investigate its meteorological effects by inducing it artificially because, inasmuch as hexagonal silver iodide nucleates ice Ih, cubic silver iodide may nucleate ice Ic. If this is so, controllable amounts may be generated in the upper atmosphere.

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References and Notes

1. A. Bravais, *J. Ec. Potytech.* **18**, 1 (1847).
2. G. Whiston, *Philos. Trans. R. Soc. London* **33**, 257 (1727).
3. A. N. Greshow, *ibid.* **45**, 524 (1748).
4. C. G. Andrus, *Mon. Weather Rev.* **43**, 213 (1915).
5. R. A. R. Tricker, *Ice Crystal Haloes* (Optical Society of America, Washington, D.C., 1979).
6. B. Kamb, in *Structural Chemistry and Molecular Biology*, A. Rich and N. Davidson, Eds. (Freeman, San Francisco, 1968), p. 507.
7. N. H. Fletcher, *The Chemical Physics of Ice* (Cambridge Univ. Press, Cambridge, 1970).
8. P. V. Hobbs, *Ice Physics* (Clarendon, Oxford, 1974).
9. E. Whalley, in *The Hydrogen Bond*, P. Schuster, G. Zundel, C. Sandorfy, Eds. (North-Holland, Amsterdam, 1976), p. 1425.
10. J. E. Bertie, L. D. Calvert, E. Whalley, *J. Chem. Phys.* **38**, 840 (1963).
11. ______, *Can. J. Chem.* **42**, 1373 (1964).
12. H. R. Pruppacher and J. D. Klett, *Microphysics of Clouds and Precipitation* (Reidel, Dordrecht, 1978), chap. 16.
13. L. Besson, *C. R. Acad. Sci.* **170**, 334 (1920).
14. J. M. Pernter and F. M. Exner, *Meteorologische Optik* (Braumüller, Vienna, 1922), p. 244.
15. W. J. Humphries, *Physics of the Air* (McGraw-Hill, New York, ed. 2, 1929), p. 483.
16. E. C. W. Goldie, G. T. Meaden, R. White, *Weather* **31**, 304 (1976).
17. R. A. R. Tricker, *J. Opt. Soc. Am.* **69**, 1093 (1979).
18. L. Besson, *C. R. Acad. Sci.* **140**, 959 (1905).
19. J. M. Pernter, *ibid.*, p. 1367.
20. Chosen as a mean of the values reported by S. LaPlaca and B. Post [*Acta Crystallogr.* **13**, 503 (1960)] and R. Brill and A. Tippe [*ibid.* **23**, 343 (1967)].
21. M. Oguro and A. Higashi, in *Physics and Chemistry of Ice*, E. Whalley, S. J. Jones, L. W. Gold, Eds. (Royal Society of Canada, Ottawa, 1973), p. 338.
22. A. Higashi, *J. Cryst. Growth* **35**, 24–25, 102 (1974).
23. ______, personal communication.
24. H. Kanno, R. J. Speedy, C. A. Angell, *Science* **189**, 880 (1975).
25. This is contribution 18825 from the National Research Council of Canada.

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Natural Disturbance and the Steady State in High-Altitude Balsam Fir Forests

Abstract. *Wind-induced, cyclic waves of death, regeneration, and maturation constantly move through the high-altitude balsam fir forests in the northeastern United States. Biomass and productivity relations, species diversity, and nutrient cycling patterns are closely tied to this cycle of disturbance. Disturbance is thus an integral part of the long-term maintenance of this ecosystem. Since forests of this type normally include all phases of the disturbance-regeneration cycle, they may constitute a steady-state ecosystem in equilibrium with the surrounding environment.*

It has been an underlying assumption of much of plant ecology that, in the absence of disturbance, ecosystems eventually reach a "climax" condition in which the structure and species composition of the vegetation do not change appreciably over time (*1*). This assumption is belied by the fact that moderate- to large-scale disturbances are omnipresent in many natural ecosystems (*2, 3*). Fires, floods, windstorms, and insect outbreaks exert powerful influences that cannot be ignored in ecosystem studies (*4*). Disturbance and the responses to it must be viewed as normal aspects of the long-term maintenance of ecosystems.

Disturbance is common in the "wave-regenerated" balsam fir (*Abies balsamea*) forest of the northeastern United States (*5, 6*). The canopy of the balsam fir forest is broken by numerous crescent-shaped strips of dead trees (Fig. 1). These trees may appear to have been flattened by strong gusts. However, closer examination shows that each area is actually a band of standing dead trees, with mature forest on one side and young, vigorously regenerating forest on the other (Fig. 2).

This pattern occurs at high elevations in the Adirondack Mountains of New York, the White Mountains of New Hampshire, and the Mount Katahdin region of Maine (*5, 6*). Similar patterns are found in *Abies veitchii* and *A. mariesii* forests in the mountains of Japan—in particular on Mount Shimagare ("mountain with dead tree strips") (*7, 8*).

Each band is actually a "wave" moving slowly through the forest, with trees dying at the wave's leading edge and being replaced by seedlings (*5, 6, 9*). These waves move in the general direction of the prevailing wind (Fig. 2) at speeds of 0.75 to 3.3 m per year (*5, 6, 8–11*). Speeds are apparently related to the degree of exposure to the prevailing wind, with the highest wave speeds occurring on or near ridgetops. Trees directly exposed to the prevailing wind are sub-

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jected to considerably higher wind velocities than trees farther back (*12*); presumably this is the cause, at least indirectly, of increased tree death at the wave's leading edge. Increased rime deposition and branch breakage in winter, winter desiccation, and summer cooling may all be involved (*5, 6, 13*).

Waves predictably follow each other at intervals of about 60 years. One possible reason for the predictable recurrence of the waves is that balsam fir, the dominant layer in the ecosystems involved, has a life-span of 60 to 80 years (*14*). Thus, about 60 years after the passage of a wave, all the trees in the area are simultaneously becoming senescent and are unusually susceptible to biotic or abiotic stress. In such a stand any localized disturbance could expand windward under the influence of the prevailing wind and develop into a wave. Since nearly all of the balsam fir stands in these areas developed after the passage of previous waves, new waves could follow 60 years behind the old ones and would be constantly moving into susceptible forests.

The steady movement of a wave through the forest results in a developmental gradient (Fig. 2). Mature forest precedes the wave, followed by a zone of dead trees with seedlings underneath, an area of very dense saplings, and a progressively older stand leading back to mature forest. This gradient is well suited to the study of processes related to ecosystem development (Table 1). We focused on changes in nutrient cycling patterns, emphasizing nitrogen, which is strongly influenced by vegetational factors (*4*).

When a wave moves through an old stand and the overstory dies, more light and heat penetrate to the forest floor. The rate of decomposition may increase, and nutrients formerly taken up by the trees accumulate in the soil or are removed by leaching. Thus the concentrations of soil nitrate are relatively high, as are the concentrations of nitrogen and potassium in new fir leaves (Table 1). The disappearance of the older fir canopy is accompanied by a flush of herbaceous growth, particularly by intolerant species such as *Solidago macrophyllum* and *Rubus idaeus*. Species richness and equitability are greatest during this phase of degeneration and regeneration (Table 1). Fir seedlings also grow rapidly, but this leads to intense competition and rapid thinning. Their density is typically reduced 90 percent in the first few years after a wave passes through.

Five to 10 years after the old overstory disappears, the fir seedlings have formed a dense stand that shades out the herb layer of the regeneration zone. The fir stand is highly productive at this point (~ 800 g/m^2-year) and accumulates living biomass and nitrogen at a high net rate (Table 1). A few fir seedlings and scattered individuals of tolerant herb species survive beneath the dense canopy; however, species richness and equitability are both extremely low during this phase, and balsam fir accounts for up to 99.9 percent of the aboveground net primary productivity.

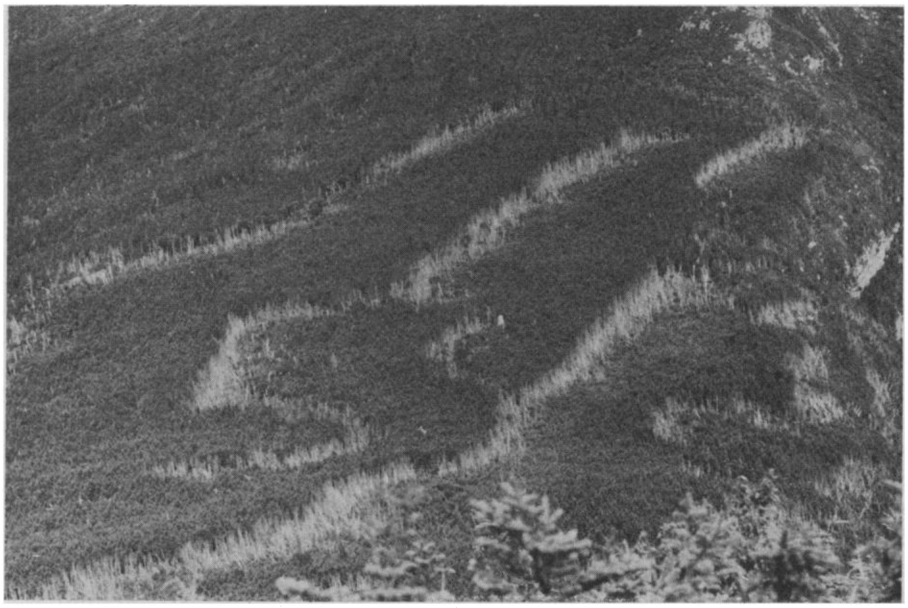

Fig. 1. Regeneration waves near Mount Katahdin, Maine.

After about 35 years the canopy begins to open. Net productivity gradually declines, dropping to about 650 g/m^2-year in the 50- to 60-year-old forest. Most of this production is directed toward replacing lost tissues, such as leaves and old branches, and the net accumulation of nitrogen in the living biomass is relatively low (Table 1). Herb productivity under the mature fir canopy averages ~ 18 g/m^2-year. Most of this growth is by tolerant species such as *Oxalis montana*, but individuals of less tolerant species may be found in the more open patches. Fir seedlings (mostly > 15 cm tall) are present in large numbers. They may survive for years under moderate shade and then grow rapidly when the canopy disintegrates.

These phases repeat with a period of about 60 years; thus the ecosystem at any fixed point is in a constant state of change as it undergoes the cycle of disturbance and regeneration. However, a wave-regenerated forest normally contains all states of development, and since the number and speed of the waves are relatively constant from year to year, the proportion of the total area in each stage tends to remain constant. This implies that ecosystem parameters such as net primary productivity, decomposition, biomass accumulation, and nutrient loss, which vary during the regeneration cycle, are also constant if averaged over a sufficiently large area (except for year-to-year variations due to weather). The ecosystem as a whole is thus in what might be termed a steady-state condition, even though particular areas appear to be constantly changing (*5*).

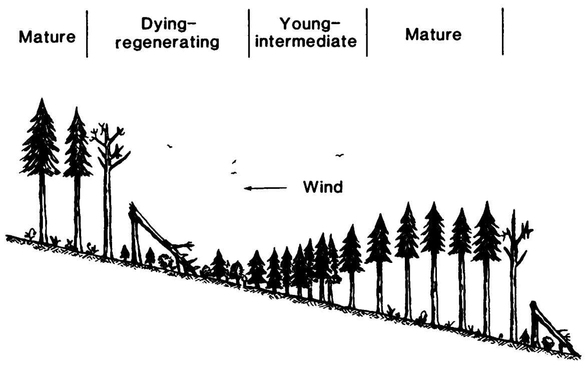

Fig. 2. Diagrammatic cross section through a regeneration wave. This pattern is associated with the bands of dead trees visible in Fig. 1.

The fact that this ecosystem appears to be in a steady state may lead one to ask whether it is also in equilibrium with its surroundings in terms of nutrients and total biomass (living biomass + dead biomass + soil organic matter). That is, over a long period of time (several cycles), do inputs equal outputs or is the system slowly accumulating or losing nutrients? Little is known about long-term biomass trends or nutrient fluxes in high-altitude fir forests, but their behavior during the disturbance cycle may be analogous to that of the spruce-hardwood ecosystems at lower elevations in the same mountains. For several years after clear-cutting, developing spruce-hardwood ecosystems undergo accelerated decomposition, increased nitrification and mineralization, and a net loss of nutrients and biomass (net losses of soil organic matter outweigh net gains in living biomass) (*15, 16*). However, the abundance of dissolved nutrients (and water) enables rapid growth of new biomass and the reestablishment of biotic control over the flux of water, nutrients, and energy through the ecosystem. As the spruce-hardwood ecosystem matures, the rate of decomposition, the concentration of substances dissolved in the soil solution, and the rate of nutrient loss in drainage water all decline. There is a net accumulation of biomass (both living and dead) and of nutrients. The cycle is repeated again after another clear-cutting.

High-altitude fir forests and other American and Scandinavian boreal forests (*4, 17*) apparently share several aspects of the spruce-hardwood cycle. Soil nitrate and the concentration of nitrogen and potassium in leaf tissues are maximal in the recently disturbed zone, suggesting increased availability of these nutrients in the soil and perhaps increased losses from leaching. The concentrations of soil nitrate and leaf tissue nitrogen are lowest in rapidly growing young fir forests where living biomass and nitrogen accumulation are greatest; the concentrations increase in the mature forest when the canopy begins to break up and the accumulation rates of nitrogen and biomass drop (Table 1). The analogy suggests that during the early phases of regeneration the high-elevation fir forest loses biomass and nutrients, while in later stages it accumulates them, but over the complete cycle (or over a large area), the gains and losses balance out (*4*). Woodmansee (*18*) suggested that there may be a similar cyclic balancing of nutrient gains and losses in semiarid grassland ecosystems.

The concept of steady state described here differs from the classic climax concept in that it fully incorporates large- or small-scale disturbance, which is seen as an integral part of the system rather than as an occasional aberration. The wave-regenerated forest is an unusually orderly one in which disturbance is omnipresent and highly predictable; however, we believe that the prevailing wind merely increases the spatial predictability and organization of a disturbance cycle that would occur in any case in these highly stressed forests. In spruce-fir forests at lower elevations, defoliating insects (*19*) may serve the same destructive function as the wind does at higher elevations, except that the ecosystem recycles itself in patches rather than waves. Similar but

Table 1. Means and ranges for some ecosystem parameters that vary during wave regeneration in fir forests on Whiteface Mountain. Biomass and productivity were estimated by standard dimension analysis techniques (*20*). The data are based on one to five plots in each phase on each of four different transects, giving a total of 30 100-m^2 plots (8 dying-regenerating, 11 young-intermediate, 11 mature). Biomass accumulation rates were calculated by fitting a second-order curve to a graph of age plotted against total biomass (to infer the development pattern of an "average" stand) and then dividing the total biomass into its component tissues. To determine the nitrogen accumulation of a typical stand, tissue samples from nine trees of various ages were analyzed (*21*). Nitrogen accumulation rates were calculated by multiplying the weight of each tissue in an average stand of a given age by the concentration of its nitrogen and determining the differences between stands of different ages. Nitrogen accumulation rates given here are thus analogous to biomass accumulation rates in that they represent yearly changes in the total nitrogen content of the stand. Herb productivity and species richness were estimated by harvesting ten 1-m^2 subplots in each of 14 plots (four dying-regenerating, five young-intermediate, five mature) located along two different transects. The Shannon-Wiener index of species diversity (*22*) was also calculated for each of these plots. Soil samples were collected in August and September 1972 at 11 points along one transect, and their ammonium and nitrate content were determined by standard techniques (*21*). The nitrate concentrations were similar on both dates, but the ammonium concentrations were about 40 percent lower in the samples taken in September. The ammonium values given are for samples collected in August. First-year fir needles collected in mid-August 1971 from sunlit branches on ten trees near each of six points along one transect were analyzed for nitrogen and potassium by standard techniques (*21, 23*). Details of the sampling and analytical techniques used are given in (*5*). Values in the same row followed by the same letter superscript are not significantly different at $P < .05$ (Student's t-test).

Measured attribute	Zone of death and regeneration	Zone of young and intermediate firs (10 to 35 years old)	Zone of mature firs (35 to 60 years old)
Living for biomass (kg/m^2)	3.0 (0.5 to 8)^a	6.2 (3 to 10)^b	10.3 (7 to 12)^c
Aboveground net primary productivity for firs (g/m^2-year)	425 (200 to 800)^a	800 (650 to 950)^b	640 (500 to 800)^c
Living biomass accumulation rate (g/m^2-year)	Negative	225 (175 to 250)	125 (90 to 175)
Nitrogen accumulation rate in living biomass (g/m^2-year)	Negative	0.75 (0.4 to 1.35)	0.13 (0.0 to 0.35)
Firs per square meter			
Overstory	8.25* (4.5 to 13)^a	5.5 (1 to 13)^a	0.45 (0.1 to 1.0)^b
Seedlings	15 (1 to 50)^a	26 (0.1 to 100)^a	82 (25 to 150)^b
Herb productivity (g/m^2-year)			
Tolerant species	33 (20 to 50)^a	4.6 (0.5 to 15)^b	16.5 (5 to 25)^c
Intolerant species	22 (5 to 50)^a	1.2 (0 to 3)^b	1.5 (0.1 to 6)^c
Total	55 (35 to 100)^a	5.8 (0.5 to 13)^b	18 (5 to 30)^c
Species per square meter	7.1 (6 to 8)^a	4.4 (2 to 7)^b	5.5 (4 to 7)^b
Shannon-Wiener index	0.18 (0.15 to 0.25)^a	0.020 (0.002 to 0.05)^b	0.065 (0.025 to 0.1)^c
Soil ammonium (ppm)	40 (35 to 45)^a	52 (40 to 70)$^{a,\,b}$	59 (45 to 80)^b
Soil nitrate (ppm)	5.3 (4 to 7)^a	2.3 (0.8 to 6.5)^b	3.9 (2 to 6.5)^a
Leaf tissue nitrogen (%)	1.84 (1.6 to 2.1)^a	1.58 (1.35 to 1.90)^b	1.69 (1.35 to 2.1)^c
Leaf tissue potassium (%)	0.76 (0.69 to 0.83)^a	0.58 (0.42 to 0.77)^b	0.64 (0.43 to 0.77)^c

*Regeneration layer.

more random steady-state patterns have been proposed for other forest ecosystems (*3, 16*). Inherent in the steady-state hypothesis is the idea that postdisturbance changes such as accelerated decomposition, mineralization, and nitrification are normal aspects of ecosystem maintenance and may even be components of a homeostatic response leading to rapid recovery.

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References and Notes

1. F. E. Clements, *J. Ecol.* **24**, 252 (1936).
2. E. W. Jones, *New Phytol.* **44**, 130 (1945); P. S. White, *Bot. Rev.* **45**, 229 (1979).
3. W. S. Cooper, *Bot. Gaz. (Chicago)* **55**, 1 (1913); A. S. Watt, *J. Ecol.* **35**, 1 (1947); H. E. Wright, *Science* **186**, 487 (1974).
4. P. M. Vitousek and W. A. Reiners, *BioScience* **25**, 376 (1975).
5. D. G. Sprugel, thesis, Yale University, New Haven, Conn. (1974).
6. ______, *J. Ecol.* **64**, 889 (1976).
7. Y. Oshima, M. Kimura, H. Iwaki, S. Kuroiwa, *Bot. Mag. Tokyo* **71**, 289 (1958); H. Iwaki and T. Totsuka, *ibid.* **72**, 255 (1959); S. Kuroiwa, *ibid.*, p. 413.
8. K. Kai, *Geogr. Rev. Jpn.* **47**, 709 (1974).
9. We studied the bands by laying transects through 16 different bands on Whiteface Mountain, New York, and on various mountains in New England. Each transect began in the mature forest uphill from the zone of death and extended to the mature forest on the other side. At 10-m intervals along each transect we chose five trees at random and determined their ages. We also periodically inspected trees at or near the wave edges and repeatedly photographed marked plots.
10. The direction of wave movement on Whiteface Mountain was estimated on the ground and then checked and corrected if necessary by referring to aerial photographs. Wave speed was estimated by calculating the slope of a graph of the age of the trees plotted against their distance from the wave edge.
11. S. Nicholson and J. T. Scott, in *Vegetation-Environment Relations at Whiteface Mountain in the Adirondacks*, J. G. Holway and J. T. Scott, Eds. (State Univ. of New York, Albany, 1969), p. 149; E. W. Holroyd, *For. Sci.* **16**, 222 (1970).
12. G. S. Raynor, *For. Sci.* **17**, 351 (1971).
13. J. Warren Wilson, *J. Ecol.* **47**, 415 (1959); R. Geiger, *The Climate Near the Ground* (Harvard Univ. Press, Cambridge, Mass., 1966), p. 350.
14. R. F. Morris, *For. Chron.* **24**, 106 (1948).
15. F. H. Bormann, G. E. Likens, D. W. Fisher, R. S. Pierce, *Science* **159**, 882 (1968); W. H. Smith, F. H. Bormann, G. E. Likens, *Soil Sci.* **106**, 471 (1968); G. E. Likens, F. H. Bormann, N. M. Johnson, D. W. Fisher, R. S. Pierce, *Ecol. Monogr.* **40**, 23 (1970); F. H. Bormann and G. E. Likens, *Sci. Am.* **223**, 92 (October 1970); A. S. Dominski, thesis, Yale University, New Haven, Conn. (1971); F. H. Bormann, G. E. Likens, T. G. Siccama, R. S. Pierce, J. S. Eaton, *Ecol. Monogr.* **44**, 255 (1974); W. W. Covington, thesis, Yale University, New Haven, Conn. (1976); G. E. Likens, F. H. Bormann, R. S. Pierce, J. S. Eaton, N. M. Johnson, *Biogeochemistry of a Forested Ecosystem* (Springer-Verlag, New York, 1977).
16. F. H. Bormann and G. E. Likens, *Pattern and Process in a Forested Ecosystem* (Springer-Verlag, New York, 1979).
17. H. Hesselman, *Medd. Statens Skogsforsoeksans.* **13–14**, 923 (1917); L. G. Romell, *Cornell Univ. Agric. Exp. Stn. Mem. 170* (1935); R. F. Wright, *Ecology* **57**, 649 (1976).
18. R. G. Woodmansee, *BioScience* **28**, 488 (1978).
19. W. J. Mattson and N. D. Addy, *Science* **190**, 515 (1975).
20. R. H. Whittaker and G. M. Woodwell, in *Productivity in Forest Ecosystems*, P. Duvigneaud, Ed. (Unesco, Paris, 1971), p. 159.
21. S. A. Wilde, S. K. Voigt, J. G. Iyer, *Soil and Plant Analysis for Tree Culture* (Oxford and IBH, New Delhi, 1972), pp. 56-63.
22. C. J. Krebs, *Ecology: The Experimental Analysis of Distribution and Abundance* (Harper & Row, New York, ed. 2, 1978), pp. 455-456.
23. G. E. Likens and F. H. Bormann, *Yale Univ. Sch. For. Bull.* **79**, (1970).
24. Supported in part by grants from the National Science Foundation to the Hubbard Brook Ecosystem Study. We thank G. E. Hutchinson, R. S. Miller, R. N. Muller, and D. M. Smith for helpful suggestions; S. MacIntyre and J. Ginsberg for assistance in the field; and the Atmospheric Sciences Research Center of the State University of New York, Albany, for the use of their facilities.

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Reactivation of an Inactive Human X Chromosome: Evidence for X Inactivation by DNA Methylation

Abstract. *A mouse-human somatic cell hybrid clone, deficient in hypoxanthine-guanine phosphoribosyltransferase (HPRT) and containing a structurally normal inactive human X chromosome, was isolated. The hybrid cells were treated with 5-azacytidine and tested for the reactivation and expression of human X-linked genes. The frequency of HPRT-positive clones after 5-azacytidine treatment was 1000-fold greater than that observed in untreated hybrid cells. Fourteen independent HPRT-positive clones were isolated and analyzed for the expression of human X markers. Isoelectric focusing showed that the HPRT expressed in these clones is human. One of the 14 clones expressed human glucose-6-phosphate dehydrogenase and another expressed human phosphoglycerate kinase. Since 5-azacytidine treatment results in hypomethylation of DNA, DNA methylation may be a mechanism of human X chromosome inactivation.*

According to the Lyon hypothesis, one of the two X chromosomes is inactivated in the somatic cells of the normal mammalian female (*1*). This event occurs early in embryonic development, and either the paternal or maternal X is randomly inactivated. Once established, the pattern of inactivation remains the same for that cell and all of its descendants. This inactivation process achieves dosage compensation between males and females for X-linked gene products. The main features of the X-inactivation theory have been amply verified by genetic, cytologic, and biochemical investigations (*2*). The female embryo initially has two active X chromosomes and one X is inactivated in somatic cells later in development (*3*). The entire human X is not inactivated, since the locus for microsomal steroid sulfatase (STS) escapes inactivation (*4–6*). The gene locus for STS is at the distal end of the short arm of the human X chromosome (*5, 7*).

Table 1. Production of HAT-resistant subclones from clone 37-26R-D after 5-azacytidine treatment. Clone 37-26R-D contains the structurally normal inactive human X chromosome. Cells were plated into 60-mm dishes (10^5 cells per dish) and 24 hours later, they were treated with 5-azacytidine at the concentration indicated. After a 24-hour exposure, the cultures were washed and maintained in normal growth medium for 72 hours. The medium was then changed to HAT (*15*), and after maintenance in HAT for 2 weeks, colonies were visible. Cells were washed with isotonic saline and fixed with methanol and acetic acid (3:1). Cells were stained with 1 percent Giemsa, and colonies were counted. The average number of colonies per treatment is derived from five dishes at each concentration.

Concentration of 5-azacytidine (μM)	HAT-resistant clones per 10^5 cells	
	Average	Range
Control	0	
0.1	0	
0.5	1	0-3
1	26	23-30
2	142	133-151
5	107	90-138
10	41	35-50

Although several models have been proposed to explain the mechanism of X inactivation at a molecular level (*8*), experimental evidence in support of these theories is lacking. One hypothesis suggests that DNA modification by methylation could be a mechanism of X inactivation (*9*); this model could be verified by modifying or reversing X inactivation by interfering with DNA methylation. The only methylated base found in mammalian DNA is 5-methylcytosine (*10, 11*), and 5-azacytidine (5-azaC) is a cytidine analog that can be integrated into DNA (*12, 13*). Incorporation of 5-azaC leads to hypomethylation of DNA (*13*), which is attributed to impaired methylation at the sites of substitution of 5-azaC as a result of the presence of nitrogen in place of carbon at the fifth position in the pyrimidine ring. Treatment of mouse fibroblasts with 5-azaC not only significantly reduces DNA methylation but also induces formation