tion than individuals in light gaps or edges. Although there is some evidence that fruit production sometimes differs between individuals in light gaps and conspecifics under closed canopy (*9*), the evolutionary basis for these differences is not yet clear.

The evolutionary basis for faster removal of fruits from light gap and edge sites remains speculative. It is doubtful that either the birds or the fruits act only as independent variables in this interaction to which the other must adjust. A disproportionate number of plant species that fruit in light gaps have brightly colored fleshy fruits in comparison to plant species that generally fruit under closed forest canopy. Brightly colored fruits may be discovered more readily in sunlit patches than under closed canopy. The attraction of frugivores to these sites may be enhanced by their learning that such sites potentially offer a concentration of fruit. Hence, concentration of fruits and frugivores in light gaps and edges may be continually reinforced in ecological and evolutionary time.

These results also emphasize that disturbances play an important role not only in interactions between temperate fruits and birds themselves, but also in the organization of temperate forest communities. In ecology "equilibrium"-thinking has clouded the potential importance of small- and large-scale disturbances in the organization of biological communities. Investigations in a number of terrestrial and aquatic systems have begun to reveal the widespread importance of disturbance in the normal organization of communities (*1*, *10*). The disturbance regime in a community affects the pattern of spatial heterogeneity, the life history adaptations of the species, and the resultant relative abundances of species. In many coniferous and deciduous forests of North America, small-scale disturbances occur on a given patch of landscape every 5 to 250 years, depending on the type of forest and its location (*1*). Large-scale disturbances in some areas occur at longer intervals. We need to know how much the life history strategies of temperate forest plants and animals are responses to disturbance as compared to steady-state conditions if we are to understand how these communities are organized.

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References and Notes

1. A. N. Auclair, and G. Cottam, *Ecol. Monogr.* **41**, 153 (1971); J. D. Henry and J. M. A. Swan, *Ecology* **55**, 772 (1974); H. S. Horn, in *Theoretical Ecology: Principles and Applications*, R. M. May, Ed. (Saunders, Philadelphia, 1976), p. 187; P. J. Grubb, *Biol. Rev.* **52**, 107 (1977); T. T. Kozlowski and C. E. Ahlgren, Eds. *Fire and Ecosystems* (Academic Press, New York, 1974); C. G. Lorimer, *Ecology* **58**, 139 (1977); P. L. Marks, *Ecol. Monogr.* **44**, 73 (1974); C. D. Oliver and E. P. Stephens, *Ecology* **58**, 562 (1977); S. T. A. Pickett, and J. N. Thompson, *Biol. Conserv.* **13**, 27 (1978); G. B. Williamson, *Ecology* **56**, 727 (1975).
2. But see, A. J. Smith, *Ecology* **56**, 19 (1975).
3. A. C. Bent, *U.S. Natl. Mus. Bull.* **195**, 1 (1948); R. R. Graber, J. W. Graber, E. L. Kirk, *Ill. Nat. Hist. Surv. Biol. Notes No. 68* (1970).
4. J. R. Carpenter, *Ecology* **16**, 203 (1935); A. C. Twomey, *Ecol. Monogr.* **15**, 173 (1945); A. C. Bent, *U.S. Nat. Mus. Bull.* **196**, 1 (1949); R. R. Graber, J. W. Graber, E. L. Kirk, *Ill. Nat. Hist. Surv. Biol. Notes No. 75* (1971); P. Williamson, *Ecol. Monogr.* **41**, 129 (1971).
5. Throughout, differences in rates were tested with analysis of covariance using $\log_{10}$ and arc sin transformations where necessary to correct for heteroscedasticity and nonlinearity of regression lines.
6. Discovery is used here loosely. We do not yet know whether certain sites from which no fruit has been removed have not been found by the birds, or whether the sites are being avoided for other reasons even though the birds know fruit is present.
7. J. N. Thompson and M. F. Willson, in preparation.
8. It is reasonable to assume that, if a plant invests energy in making fleshy fruits to be taken by dispersal agents, there is a selective advantage in having seeds dispersed.
9. L. K. Halls and R. Alconiz, *J. Wildl. Manage.* **32**, 185 (1968); L. K. Halls, *U.S. For. Serv. Res. Pap. SE No. 90* (1973); I. V. Hall, and J. D. Sibley, *Can. J. Plant Sci.* **56**, 885 (1976).
10. P. K. Dayton, *Ecol. Mongr.* **41**, 351 (1971); M. L. Heinsel, *U.S. For. Serv. Pac. Northwest For. Range Exp. Stn. Res. Notes* (1971), pp. 61–72; J. R. Karr, *Ecol. Monogr.* **46**, 457 (1976); S. A. Levin, *Annu. Rev. Ecol. Syst.* **7**, 287 (1976)); S. T. A. Pickett, *Am. Nat.* **110**, 107 (1976); P. J. Regal, *Science* **197**, 622 (1977); P. F. Sale, *Am. Nat.* **111**, 337 (1977), J. A. Wiens, *Annu. Rev. Ecol. Syst.* **7**, 31 (1976); *Am. Sci.* **65**, 590 (1977).
11. We thank F. A. Bazzaz, G. Brown, R. Condit, D. H. Janzen, J. R. Karr, S. T. A. Pickett, J. F. Thompson and an anonymous reviewer for comments on an earlier draft. Supported in part by a grant-in-aid of research from Sigma Xi.

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Absence of Adenosine Deaminase Activity in a Mammalian Cell Line Transformed by Rous Sarcoma Virus

Abstract. *No detectable adenosine deaminase activity was found in whole cells or 105,000g cytosol preparations of B-mix K-44/6 cells when either [^{3}H]adenosine or [^{3}H]arabinosyladenine was used as substrate. When grown in tissue culture medium supplemented with horse serum these cells provide a deaminase-free system not requiring the use of an adenosine deaminase inhibitor.*

Adenosine deaminase (adenosine aminohydrolase, E.C. 3.5.4.4) has a very wide distribution in animal tissues and has been studied extensively [see (*1*)]. In addition to adenosine, the enzyme accepts a wide variety of adenine nucleosides as substrates, including deoxyadenosine and arabinosyladenine (ara-A) (*2*). Multiple forms of adenosine deaminase may be found even within a single tissue, and the patterns of inheritance of its isoenzymes have been studied (*3*). The enzyme participates in the catabolism of adenine nucleosides, resulting in the production of hypoxanthine for use in purine salvage pathways. In the heart, this enzyme may serve an important regulatory function by inactivating adenosine, a potent vasodilator (*4*). A deficiency of adenosine deaminase has been associated with severe combined immunodeficiency disease (SCID) in children (*5*), but a well-defined causal relationship has not been established (*6*). Residual adenosine deaminase activity has been detected in the fibroblasts derived from patients with SCID and adenosine deaminase deficiency (*7*).

Studies in vitro on the mode of action of the antiviral drug ara-A are complicated by the deamination of ara-A to arabinosylhypoxanthine (ara-H) by adenosine deaminase (*8*). Although the adenosine deaminase activity associated with the serum normally used to supplement tissue culture media can be minimized by prolonged thermal inactivation (*9*), or through the use of a variety of adenosine deaminase inhibitors (*10*), a cell line devoid of adenosine deaminase activity has not been described. We describe in this report an established line of mammalian cells (B-mix K-44/6) totally lacking detectable adenosine deaminase activity. The B-mix K-44/6 cells were subcloned from a population of embryonic rat cells transformed in vitro by the Prague strain of Rous sarcoma virus (*11*). These large epithelioid cells are malignant for homologous rat hosts and have been shown both by experiments in vivo (*11*) and by cell fusion studies with chick-embryo fibroblasts (*12*) to contain the genome of Rous sarcoma virus. In our laboratory B-mix K-44/6 cells have proved useful in elucidating the effects of ara-A on macromolecular biosynthesis in a cell system not requiring the use of an inhibitor of adenosine deaminase (*13*).

In this study we used, in addition to B-mix K-44/6 cells, an established line of human cells derived from an epidermoid carcinoma of the mouth (KB) and a continuous line of baby hamster kidney cells

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Cell line	Activity	
	[^{3}H]Adenosine	[^{3}H]Ara-A
KB	22.7 ± 0.02	2.52 ± 0.68
BHK-21/4	8.21 ± 4.08	1.46 ± 0.28
B-mix K-44/6	<0.004	<0.004

Table 1. Adenosine deaminase activity in cytosol preparations of KB, BHK-21/4, and B-mix K-44/6 cells. Cells were planted in 75-cm^2 plastic flasks (Falcon) in Eagle's minimal essential medium (Hanks balanced salt base) supplemented with either 10 percent extensively heated calf serum (*9*) or 10 percent horse serum. When the cells reached confluency they were harvested with 0.02 percent EDTA and 0.05 percent trypsin in a Hepes buffered (*26*) balanced salt solution [HBS; see (*15*)] and washed three times in HBS (*p*H 7.4). The cells were resuspended in 11.5 ml of 25 m*M* sodium phosphate buffer (*p*H 7.0) and allowed to swell for 15 minutes. They were then homogenized by the Dounce method (55 strokes with a tight-fitting pestle), and the homogenate was clarified by centrifugation at 20,000*g* in a Beckman J-21 centrifuge for 15 minutes at 0°C. The supernatant fluids were removed, centrifuged in a Ti-50 rotor at 105,000*g* in a Beckman L3-50 ultracentrifuge for 60 minutes at 4°C, and dialyzed twice against 100 volumes of cold 25 m*M* sodium phosphate buffer (*p*H 7.0). Protein was determined according to the method of Lowry *et al.* (*27*). Reaction mixtures consisted of 1 mg of protein of the cytosol preparation to be tested, and 100 μM radioactively labeled substrate in a final volume of 1.0 ml of 25 m*M* sodium phosphate buffer (*p*H 7.0). Reactions were allowed to proceed at 37°C. Final specific activities of [^{3}H]adenosine and [^{3}H]ara-A were 0.02 Ci/mmole. At selected times from zero to 240 minutes, 50-μl portions were removed and added to two volumes of ice-cold ethanol. Chromatographic and liquid scintillation spectrometric procedures were as described in Fig. 1. The activity values are expressed as nanomoles of substrate degraded per minute per milligram of protein; each value represents the arithmetic mean of three or four determinations of initial rate plus or minus the standard error of the mean.

(BHK-21/4) as known positive controls. The provenance and culture requirements of these cells have been published (*14, 15*).

Adenosine deaminase activity was determined in these three cell lines by the use of both tritiated adenosine ([2-^{3}H]-adenosine, 20 to 25 Ci/mmole; Amersham/Searle) and tritiated arabinosyladenine ([2-^{3}H]ara-A, 10 to 20 Ci/mmole; New England Nuclear) as substrates.

Rates of deamination of ara-A and adenosine were compared for KB, BHK-21/4, and B-mix K-44/6 cells grown in monolayer cultures in a medium essentially devoid of adenosine deaminase activity (Fig. 1). Whereas 100 μM ara-A was deaminated to ara-H with a half-life of 2 hours in KB cells and 3 hours in BHK-21/4 cells, no deamination could be detected after a 53-hour incubation period with B-mix K-44/6 cells when either ara-A or adenosine were used as substrates.

To ensure that the apparent lack of adenosine deaminase activity seen with B-mix K-44/6 cells did not reflect a transport phenomenon, we prepared soluble cell extracts from the three cell lines. Although deaminase activity was observed in the cytosol preparations from KB and BHK-21/4 cells, no activity was seen in extracts of B-mix K-44/6 cells (Table 1) at the practical limit of sensitivity of the assay (a 1 percent change in substrate concentration over 240 minutes). Separate determinations were made to ensure that the enzyme was not present in limiting concentrations. The final product was ara-H when ara-A was used as the substrate, whereas inosine and hypoxanthine were products when adenosine was used as substrate.

The absence of detectable adenosine deaminase activity in B-mix K-44/6 cells cannot be explained at this time. Adenosine is normally catabolized in mammalian cells by deamination to inosine. After deamination, mammalian purine-nucleoside phosphorylase (E.C. 2.4.2.1) cleaves inosine to yield hypoxanthine and ribose-1-phosphate, which may be reutilized by the cell in several metabolic pathways, including nucleotide biosynthesis (*16, 17*). Although inosine, xanthosine, guanosine, and some deoxyribosyl and some fraudulent nucleosides are substrates for mammalian purine-nucleoside phosphorylase, adenosine generally is not regarded as a physiologically significant substrate for the enzyme (*17, 18*). An alternative pathway resulting in the degradation of adenosine involves the deamination of adenylate by AMP (adenosine monophosphate) aminohydrolase (E.C. 3.5.4.6; AMP deaminase) and the subsequent dephosphorylation of inosinate to inosine. Although this pattern of adenylate catabolism occurs in other cell lines [see, for example (*19*)], its possible role in B-mix K-44/6 cells has not been explored.

Chiang and co-workers have reported (*20*) that adenosine deaminase activity in chick embryo fibroblasts was substantially reduced after infection and transformation by Rous sarcoma virus. In contrast, XC cells (*21*) (a cell line derived from a rat tumor induced by the Prague strain of Rous sarcoma virus) actively deaminate adenosine (*22*). Thus the possible relation between malignant transformation by Rous sarcoma virus and reduction or elimination of adenosine deaminase activity also remains to be elucidated.

The explanation that an inhibitor of adenosine deaminase is produced in B-mix K-44/6 cells does not appear to be valid, because extracts of these cells did not inhibit a commercial preparation of adenosine deaminase (*23, 24*).

Recently, there has been a surge of interest in the use of inhibitors of adenosine deaminase because this enzyme has a key role in the inactivation of many adenosine analogs of chemotherapeutic value. The use of such inhibitors in vitro (and perhaps even more importantly in vivo), however, is complicated by the in-

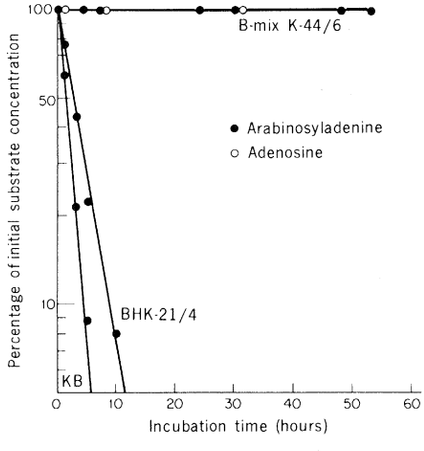

Fig. 1. Deamination of [^{3}H]arabinosyladenine or [^{3}H]adenosine by cultures of B-mix K-44/6, BHK-21/4, or KB cells. Cells were planted in 25-cm^2 plastic flasks (Falcon) in Eagle's minimal essential medium (Earle's balanced salt base) supplemented with either 5 percent extensively heated calf serum (*9*) or 5 percent horse serum. At the times indicated 200-μl portions of the media were removed and immediately added to two volumes of ice-cold ethanol to precipitate protein. All samples were stored at −20°C. Upon completion of the experiment, the samples were retrieved from the freezer and centrifuged at 700*g* for 5 minutes at 0°C in an International PR-6 centrifuge. The supernatant fluids were removed, and 5-μl portions were applied together with reference compounds to precoated (silica gel GF) thin-layer chromatography plates (Analtech). The chromatographic systems used to separate inosine, hypoxanthine, adenosine, and adenine or arabinosylhypoxanthine, arabinosyladenine, hypoxanthine, and adenine have been described (*28*). The reference compounds were detected on the developed plates by quenching with ultraviolet light at 254 nm. Radioactive metabolites were detected by scraping 0.5- or 1.0-cm-wide sections of the plate into counting vials, extracting with 0.1*N* HCl in methanol, adding 13 ml of 0.3 percent PPO (2,5-diphenyloxazole) in toluene, and counting in a Beckman LS 8100–Texas Instruments 733ASR liquid scintillation spectrometer system. Disintegrations per minute were calculated by means of a computer program [SUPERLIQ; see (*29*)] according to the compton edge method described by Horrocks (*30*). Both [^{3}H]arabinosyladenine (•) and [^{3}H]adenosine (○) were used as substrates.

teractions of these potent inhibitors with other enzymes of purine nucleoside or nucleotide metabolism. It has been shown, for instance, that both coformycin and deoxycoformycin inhibit AMP deaminase with respective K_i (inhibition constant) values of approximately $5 \times 10^{-8}M$ and $3 \times 10^{-6}M$ (*25*).

Cells of the line B-mix K-44/6 can be readily adapted to growth in medium containing horse serum. Although equine red blood cells, plasma, and serum do contain low but significant levels of adenosine deaminase activity, this activity is not detectable when the serum is used to supplement the medium at the usual level of 5 percent or 10 percent. Thus, B-mix K-44/6 cells grown in medium supplemented with horse serum provide a cell system virtually devoid of adenosine deaminase activity.

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References and Notes

1. C. L. Zielke and C. H. Suelter, in *The Enzymes*, P. D. Boyer, Ed. (Academic Press, New York, 1971), vol. 4, p. 47.
2. J. L. York and G. A. LePage, *Can. J. Biochem.* **44**, 331 (1966).
3. V. R. Potter, *Nucleic Acid Outlines* (Burgess, Minneapolis, 1960), p. 217.
4. R. M. Berne, *Physiol. Rev.* **44**, 1 (1964).
5. E. R. Giblett, J. E. Anderson, F. Cohen, B. Pollara, H. J. Meuwissen, *Lancet* **1972-II**, 1067 (1972); H. J. Meuwissen, R. T. Pickering, B. Pollara, I. H. Porter, *Combined Immunodeficiency Disease and Adenosine Deaminase Deficiency* (Academic Press, New York, 1976).
6. G. C. Mills *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **73**, 2867 (1976); D. Perrett and B. Dean, *Biochem. Biophys. Res. Commun.* **77**, 374 (1977).
7. R. Hirschhorn, N. Beratis, A. Rosen, *Proc. Natl. Acad. Sci. U.S.A.* **73**, 213 (1976).
8. S. S. Cohen, *Med. Biol.* **54**, 299 (1976).
9. P. M. Schartz, C. Shipman, Jr., R. H. Carlson, J. C. Drach, *Antimicrob. Agents Chemother.* **5**, 337 (1974).
10. H. Nakamura *et al.*, *J. Am. Chem. Soc.* **96**, 4327 (1974); P. W. K. Woo, H. W. Dion, S. M. Lang, L. F. Dahl, I. J. Durham, *J. Heterocycl. Chem.* **11**, 641 (197→ H. J. Schaeffer and C. F. Schwender, *J. Med. Chem.* **17**, 6 (1974).
11. J. Svoboda, P. Vesely, L. Donner, *Folia Biol. (Prague)* **13**, 40 (1967).
12. C. Shipman, unpublished data.
13. C. Shipman, Jr., S. H. Smith, J. C. Drach, *Proc. Natl. Acad. Sci. U.S.A.* **69**, 1753 (1972); J. C. Drach, J. N. Sandberg, C. Shipman, Jr., *J. Dent. Res.* **56**, 275 (1977).
14. C. Shipman, Jr., S. H. Smith, R. H. Carlson, J. C. Drach, *Antimicrob. Agents Chemother.* **9**, 120 (1976).
15. C. Shipman, Jr., *Proc. Soc. Exp. Biol. Med.* **140**, 305 (1969).
16. J. F. Henderson and A. R. P. Paterson, *Nucleotide Metabolism, An Introduction* (Academic Press, New York, 1973), pp. 136–151.
17. A. W. Murray, D. C. Elliot, M. R. Atkinson, *Prog. Nucleic Acid Res. Mol. Biol.* **10**, 87 (1970).
18. M. Friedkin and H. Kalckar, in *The Enzymes*, P. D. Boyer, H. Lardy, K. Myrback, Eds. (Academic Press, New York, ed. 2, 1961), vol. 5, p. 237; R. E. Parks, Jr., and R. P. Agarwal, in *The Enzymes*, P. D. Boyer, Ed. (Academic Press, New York, ed. 3, 1972), vol. 7, p. 483; M. A. Thomas, C. Shipman, Jr., J. N. Sandberg, J. C. Drach, *In Vitro* **13**, 502 (1977).
19. K. Overgaard-Hansen, *Biochim. Biophys. Acta* **104**, 330 (1965).
20. P. K. Chiang, G. L. Cantoni, D. A. Ray, J. P. Bader, *Biochem. Biophys. Res. Commun.* **78**, 336 (1977).
21. J. Svoboda, P. P. Chyle, D. Simkovic, I. Hilgert, *Folia Biol. (Prague)* **9**, 77 (1963).
22. L. Bennett, W. Shannon, P. Allan, unpublished data.
23. Adenosine deaminase from calf intestine (Boehringer, Mannheim).
24. P. Borondy and A. Glazko, unpublished data.
25. R. P. Agarwal and R. E. Parks, Jr., *Biochem. Pharmacol.* **26**, 663 (1977).
26. Hepes buffer is 4-(2-hydroxyethyl)-1-piperazine ethane sulfonate.
27. O. H. Lowry, N. J. Rosebrough, A. L. Farr, R. J. Randall, *J. Biol. Chem.* **193**, 265 (1951).
28. J. C. Drach and J. M. Novack, *Anal. Biochem.* **52**, 633 (1973).
29. P. M. Schwartz, thesis, University of Michigan (1975) [University Microfilms, Ann Arbor, Mich. (Diss. Abstr. 36, 2708B) (1975)].
30. D. L. Horrocks, *Applications of Liquid Scintillation Counting* (Academic Press, New York, 1974).
31. We thank J. M. Sandberg and M. A. Thomas for skillful technical assistance, P. M. Schwartz for preliminary data on adenosine deaminase activity in KB and BHK-21/4 cells, and M. L. Sweet for fresh horse blood. This work was funded by PHS grant DE 02731 from the National Institute of Dental Research.

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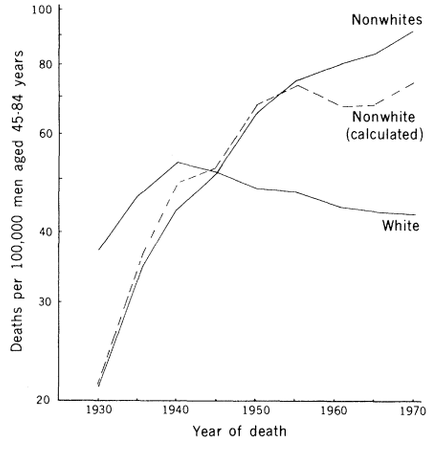
Cohort Mortality for Prostatic Cancer Among United States Nonwhites

Abstract. *In recent decades, age-adjusted mortality rates from prostatic cancer have risen precipitously among blacks, remaining unchanged among whites. It is now the most common cancer among United States black males. When nonwhite mortality rates were examined by age and birth cohort, it was found that peak rates occurred at every age in the cohort of 1896 to 1900, and declined thereafter. This presages an arrest and reversal of the time trend in summary mortality rates as more recent nonwhite cohorts reach the ages of maximum risk.*

Although prostatic cancer is the second most common cancer among men in the United States and is the most common cancer in black males, its etiology remains essentially unknown. The most striking aspect of its epidemiology is the substantially higher occurrence of the disease among blacks when compared to whites, a difference which appears not to be due to readily identifiable factors associated with socioeconomic status (*1*). Age-adjusted prostatic cancer incidence and mortality rates among blacks in the United States have increased dramatically over the past few decades (*2*). In Fig. 1, age-adjusted prostatic cancer death rates are shown for U.S. whites and nonwhites over the period 1930 to 1970. The rates have been standardized to the 1950 total U.S. male population aged 45 through 84 years. For both races, mortality increased during the decade 1930 to 1940, after which time the rates for whites declined slightly while those for nonwhites continued to increase. The approximate linearity on the logarithmic scale shows that the relative increase in the rates for nonwhites and the decrease in the rates for whites since 1940 have been essentially constant.

The sharply rising mortality from prostatic cancer among blacks raises concerns about even higher disease rates in the future. However, age-adjusted rates for various time periods often mask underlying patterns within particular age



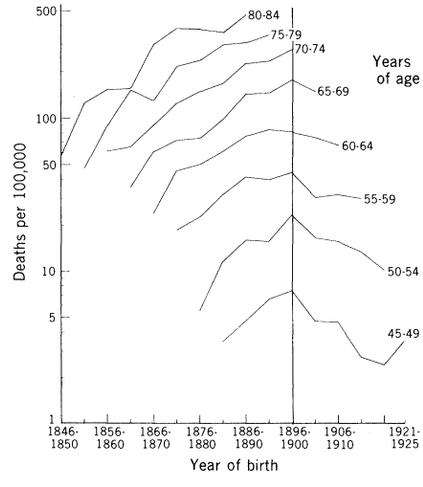


Fig. 1 (left). Annual age-adjusted prostatic cancer mortality rates per 100,000 U.S. males aged 45 to 84 years. The data are separated for whites and nonwhites, and a computed rate is shown for nonwhites. The rates are shown at 5-year intervals from 1930 to 1970 and are adjusted to the 1950 U.S. male population aged 45 to 84 years (*6*). Fig. 2 (right). Annual age-specific prostatic cancer mortality rates per 100,000 by cohort for U.S. nonwhite males born between 1846 and 1925 (*6*).

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