receptor on a presynaptic retinal terminal. These findings suggest that the retinal neurotransmitter may not be ACh, but rather that ACh modulates synaptic transmission in the visual system. This idea is supported by recent suggestions that the choline *O*-acetyltransferase present in the layers of retinal projection of the goldfish tectum is not lost after an eye is removed (*16, 17*). An attractive alternative for the cholinergic input to the optic tectum has been suggested by Gruberg and collaborators (*18, 19*). They have shown, in the turtle and the frog, that the nucleus isthmi projects to the optic tectum and that 90% of the choline *O*-acetyltransferase activity is lost after a bilateral lesion of the nuclei isthmi. One possibility is that fibers from the nucleus isthmi form nicotinic cholinergic axoaxonal synapses on retinal terminals and play a role in the control of retinotectal transmission. As suggested by Freeman (*10*) and Schmidt (*20*), the modulation of this receptor, now localized to the presynaptic terminal, may have important implications for the plasticity of synaptic connections in the goldfish visual system.

REFERENCES AND NOTES

1. S. J. Tzartos and J. M. Lindstrom, *Proc. Natl. Acad. Sci. U.S.A.* **77**, 755 (1980).
2. S. J. Tzartos, D. E. Rand, B. L. Einarson, J. M. Lindstrom, *J. Biol. Chem.* **256**, 8635 (1981).
3. L. W. Swanson *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **80**, 4532 (1983).
4. P. B. Sargent, S. H. Pike, L. Tsavaler, J. M. Lindstrom, *Soc. Neurosci. Abstr.* **10**, 935 (1984).
5. J. M. Henley *et al.*, *Brain Res.* **364**, 405 (1986).
6. R. E. Oswald, J. T. Schmidt, J. J. Norden, J. A. Freeman, *ibid.* **187**, 113 (1980).
7. J. T. Schmidt and J. A. Freeman, *ibid.*, p. 129.
8. J. A. Freeman, J. T. Schmidt, R. E. Oswald, *Neuroscience* **5**, 929 (1980).
9. N. Schechter, A. Francis, D. G. Deutsch, M. S. Gazzaniga, *Brain Res.* **166**, 57 (1979).
10. J. A. Freeman, *Nature (London)* **269**, 218 (1977).
11. R. E. Oswald and J. A. Freeman, *J. Neurochem.* **37**, 1586 (1981).
12. I. G. McQuarrie and B. Grafstein, *Brain Res.* **235**, 213 (1982).
13. R. E. Oswald and J. A. Freeman, *Neuroscience* **6**, 1 (1980).
14. M. Schwartz, D. Axelrod, E. L. Feldman, B. W. Agranoff, *Brain Res.* **194**, 171 (1980).
15. A. J. C. Östberg and J. J. Norden, *ibid.* **168**, 441 (1979).
16. N. Tumosa, W. K. Stell, F. Eckenstein, *Soc. Neurosci. Abstr.* **10**, 575 (1984).
17. C. D. Ross and D. A. Godfrey, *ibid.*, p. 575.
18. A. J. Ricciuti and E. R. Gruberg, *ibid.*, p. 574.
19. P. H. Desan, E. R. Gruberg, F. Eckenstein, *ibid.*, p. 575.
20. J. T. Schmidt, *J. Neurophysiol.* **53**, 237 (1985).
21. D. C. Thomas *et al.*, *Am. J. Epidemiol.* **122**, 1080 (1985).

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Aminoguanidine Prevents Diabetes-Induced Arterial Wall Protein Cross-Linking

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Age-associated increases in collagen cross-linking and accumulation of advanced glycosylation products are both accelerated by diabetes, suggesting that glucose-derived cross-link formation may contribute to the development of chronic diabetic complications as well as certain physical changes of aging. Aminoguanidine, a nucleophilic hydrazine compound, prevented both the formation of fluorescent advanced nonenzymatic glycosylation products and the formation of glucose-derived collagen cross-links in vitro. Aminoguanidine administration to rats was equally effective in preventing diabetes-induced formation of fluorescent advanced nonenzymatic glycosylation products and cross-linking of arterial wall connective tissue protein in vivo. The identification of aminoguanidine as an inhibitor of advanced nonenzymatic glycosylation product formation now makes possible precise experimental definition of the pathogenetic significance of this process and suggests a potential clinical role for aminoguanidine in the future treatment of chronic diabetic complications.

CROSS-LINKING OF LONG-LIVED PROteins such as collagen increases as a function of age in both animals and man. In diabetes, the rate at which this age-associated increase in collagen cross-linking occurs is greatly accelerated (*1–4*). These observations have prompted a number of investigators to hypothesize that increased cross-linking of collagen and other extracellular matrix components may be responsible, in part, for the development of some of the physical changes that occur with normal aging and for some of the chronic complications of diabetes. The mechanism by which both diabetes and aging cause increased collagen cross-linking has not been elucidated, but recent work with model proteins suggests that advanced nonenzymatic glycosylation products could function as glucose-derived collagen cross-links (*5–10*).

The sequence of nonenzymatic glycosylation product formation that leads to cross-linking of proteins is shown in Fig. 1. Initially, glucose reacts with protein amino groups via nucleophilic addition to form a chemically reversible Schiff base adduct, which subsequently rearranges to the more stable but still chemically reversible Amadori product (*11–13*). Amadori products then slowly undergo a series of further reactions with amino groups on other proteins to form glucose-derived, intermolecular cross-links such as the recently characterized advanced glycosylation product, 2-(2-furoyl)-4(5)-(2-furanyl)-1*H*-imidazole (*9, 13*). In contrast to early glycosylation products, which reach equilibrium levels after only 6 weeks, advanced glycosylation products continue to accumulate on long-lived proteins such as collagen over long periods of time (*13*). In collagen from diabetic individuals, this age-associated accumulation of advanced glycosylation products is accelerated by long-term exposure to elevated glucose concentrations (*14*).

Reasoning that glucose-derived protein cross-link formation would be prevented if reactive carbonyls on early glycosylation products could be pharmacologically blocked, we investigated the effects of a nucleophilic hydrazine compound, aminoguanidine (*15, 16*), on this process (Fig. 1). In this report we describe experiments demonstrating that aminoguanidine inhibits advanced glycosylation product formation and glucose-derived collagen cross-linking in vitro. The results also show that aminoguanidine administered to rats inhibits diabetes-induced accumulation of advanced glycosylation products and abnormal cross-linking of arterial wall connective tissue protein.

The effect of aminoguanidine on advanced glycosylation product formation was evaluated by measuring specific fluorescence, as previously described for collagen (*14*). In each sample, incorporation of repurified ^{14}C-labeled glucose into acid-precipitable protein was also determined. Under these conditions, the values obtained for total glucose incorporation are essentially

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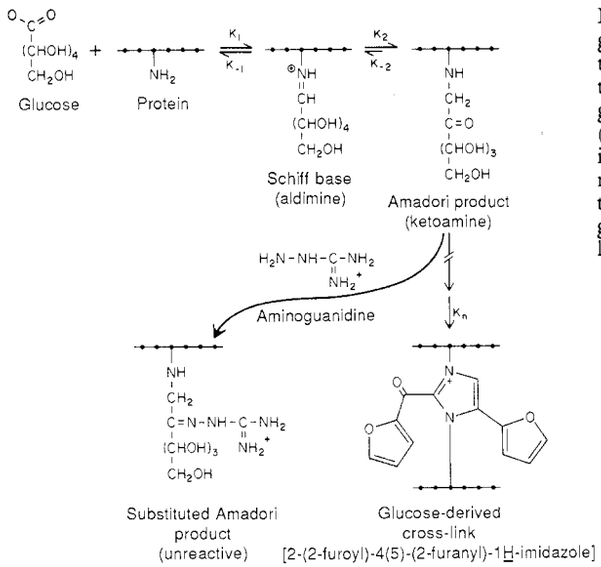

Fig. 1. Sequence of nonenzymatic glycosylation product formation that leads to cross-linking of proteins through irreversible advanced glycosylation products such as 2-(2-furoyl)-4(5)-(2-furanyl)-1*H*-imidazole. Prevention of glucose-derived protein cross-linking by reaction of aminoguanidine with early glycosylation products is shown at lower right.

equivalent to the amount of Amadori product formed (*11*, *17*). Incubation of albumin over a period of 2 weeks in the absence of glucose did not result in the formation of advanced glycosylation products (Fig. 2). In contrast, when albumin was incubated with glucose, advanced glycosylation product formation was already evident at 0.5 week, and the amount increased progressively with incubation time. Identical albumin and glucose incubation mixtures containing aminoguanidine, however, showed a 90% inhibition of advanced glycosylation product formation, while the amount of Amadori product formed remained essentially unchanged.

The effect of aminoguanidine on collagen cross-linking in vitro was evaluated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) of cyanogen bromide cleavage products of native collagen fibrils (*18*). Insoluble native fibrils were formed from acid-soluble calf skin collagen and incubated with glucose (200 m*M*) in phosphate-buffered saline, *p*H 7.4, at 37°C for 3 weeks (Fig. 3A). We chose this concentration of glucose to accelerate the formation of reactive advanced glycosylation products over the short incubation period we used, since it is the integral of glucose concentration over time that determines the extent of advanced glycosylation product accumulation (*13*). In vivo, with concentrations of glucose found in diabetic plasma (10 to 20 m*M*), this process would occur over a much longer period, consistent with the clinical time course over which pathologic changes develop in chronic diabetes. After dialysis, collagen samples were cleaved at methionine residues with CNBr, and identical amounts of protein were subjected to electrophoresis in SDS/mercaptoethanol. With time, the gel pattern of CNBr-digests from the collagen incubated with glucose showed increasing amounts of high molecular weight cross-linked peptides, including significant amounts of material that failed to penetrate the 4% stacking gel. When collagen fibrils were incubated for 3 weeks with varying concentrations of glucose (0 to 200 m*M*), a similar progression was observed in the gel patterns (Fig. 3B). As glucose concentration increased, the amount of high molecular weight peptides in the digest also increased, although even at the lowest glucose concentration (50 m*M*) a significant amount of material was unable to penetrate the stacking gel. The presence of aminoguanidine in the 3-week incubation mixture of collagen fibrils and the highest concentration of glucose, however (Fig. 3C, lane c), prevented formation of nearly all the high molecular weight (>116K) cross-linked material seen in digests of identical incubations of collagen with glucose alone (Fig. 3C, lane b). In addition, aminoguanidine also prevented the formation of several peptide bands in the molecular weight range of 30 to 50K, which appeared after incubation of collagen in buffer alone for 3 weeks at 37°C (Fig. 3C, lane a). Since these bands were faint when CNBr digests of unincubated collagen were subjected to SDS-PAGE, they probably represent glucose-derived cross-links that arose during the 3-week incubation from preexisting collagen Amadori products (*19*).

The in vivo effect of parenterally administered aminoguanidine on the formation of aortic fluorescent advanced nonenzymatic glycosylation products and aortic connective tissue cross-linking was assessed in diabetic and nondiabetic rats (Table 1). All animals received daily intraperitoneal injections of either sterile saline alone or sterile saline containing aminoguanidine (25 mg/kg-day), for 16 weeks. The amount of aortic fluorescent advanced nonenzymatic glycosyl-

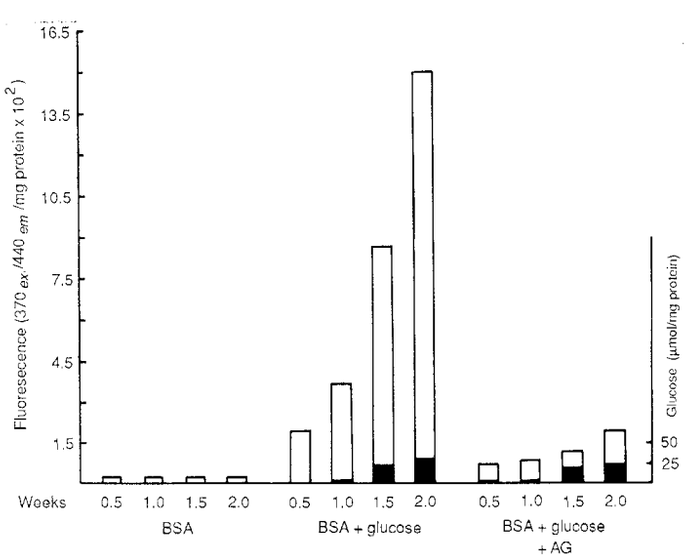

Fig. 2. Effect of aminoguanidine on advanced glycosylation product formation. Bovine serum albumin (RIA grade, Sigma) was dissolved at a concentration of 100 mg/ml in phosphate buffer (*p*H 7.4, 0.5*M* Na) alone, buffer plus D-glucose (200 m*M*), or buffer plus D-glucose plus aminoguanidine-HCl (200 m*M*). ^{14}C-Labeled glucose (9.0 μCi; specific activity 14.4 mCi/mmol; New England Nuclear) was added to each glucose-containing incubation after removal of reactive contaminants by the method of Bunn (*17*). Sodium azide (3 m*M*) was added to prevent bacterial growth. All samples were incubated at 37°C. At the indicated times, aliquots were removed for determination of specific advanced glycosylation product fluorescence as described previously (*14*), by means of a Perkin-Elmer 204 spectrofluorimeter. Protein content was assayed by the method of Bradford (*22*), and results expressed as fluorescence (measured as excitation and emission wavelengths at 370 and 440 nm, respectively) per milligram of protein $\times 10^2$ (open bars). The means (n = 3) are indicated but the errors for these samples (≤0.03) were too small to be visible on this scale. Aliquots of each sample were also taken for determination of protein-bound glucose. After precipitation with trichloroacetic acid and centrifugation, the protein pellet was washed three times with buffer and counted in a Tricarb scintillation counter (Packard Scientific). Results are expressed as micromoles of glucose per milligram of protein (closed bars).

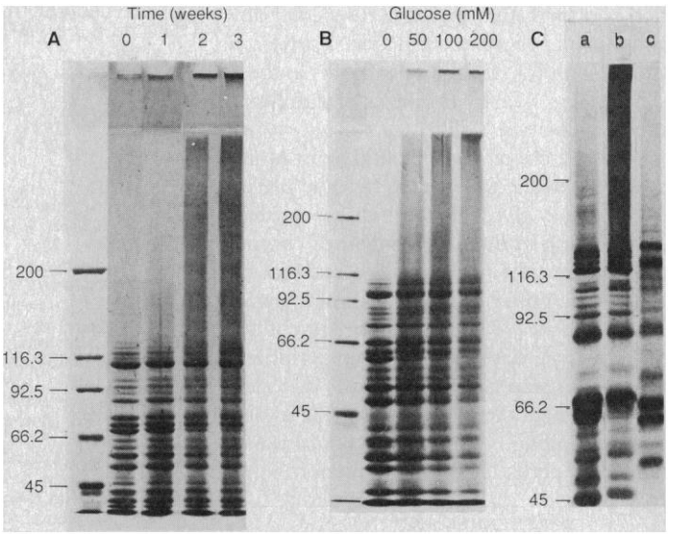

Fig. 3. Effect of aminoguanidine on collagen cross-linking in vitro. (A) Calf skin collagen (type I, Elastin Products, Pacific, MO) prepared according to the method of Gallop and Seifter (*23*) was solubilized in 0.5*M* acetic acid and purified of high molecular weight aggregates by adding NaCl to a concentration of 0.6*M* (*24*). The supernatant was then dialyzed against four changes of 0.02*M* sodium phosphate buffer, *p*H 7.4, at 4°C for 24 hours to form native collagen fibrils (*25*). Fibrils were incubated at 37°C in phosphate buffer (*p*H 7.4, 0.15*M* Na) with D-glucose (200 m*M*) for 3 weeks, and aliquots were removed for analysis at the indicated times (0, 1, 2, and 3 weeks). Prior to being subjected to SDS-PAGE, samples were dialyzed against buffer to remove glucose and then lyophilized. The lyophilized samples were rehydrated in distilled water at 60°C for 1 hour. Formic acid was then added to a final concentration of 70% (v/v). Cyanogen bromide (Pierce Chemical, analytical grade) was added in acetonitrile in a ratio of 20:1 (w/w), and cleavage was carried out at 30°C for 18 hours (*18*). Aliquots were taken for protein determination by the method of Last (*26*), and the remaining samples were dialyzed against 0.125*M* tris buffer, *p*H 6.8, containing 2% SDS and 2% glycerol, in order to prevent protein aggregation during acid removal. Equal amounts of each sample were then subjected to SDS-PAGE on 5 to 10% gradient gels with a 4% stacking gel (*27*). Gels were stained with Coomassie blue dye (*28*). (B) Collagen samples prepared as described above were incubated for 3 weeks in buffer containing 0, 50, 100, and 200 m*M* concentrations of glucose. After incubation, collagen samples were treated with CNBr and analyzed by SDS-PAGE. (C) Collagen samples prepared as described above were incubated for 3 weeks at 37°C in either buffer alone (lane a), buffer plus 200 m*M* glucose (lane b), or buffer, 200 m*M* glucose, and 200 m*M* aminoguanidine (lane c). Following incubation, collagen samples were treated with CNBr, analyzed by SDS-PAGE, and stained with silver stain (*29*).

ation product formed was determined as described in the legend to Fig. 2, after complete solubilization of tissue samples by pepsin and proteinase K. The degree of aortic connective tissue cross-linking was assessed by three different procedures: (i) solubility in 0.5*M* acetic acid, a standard method that solubilizes non–cross-linked collagen molecules and those cross-linked by aldimine-containing, lysyl oxidase–generated cross-links (*20*); (ii) CNBr digestion, a methionine-specific cleavage procedure that generates a standard and well-characterized series of soluble collagen fragments (*21*); and (iii) degradation by pepsin, a procedure that selectively degrades the only two sites on each collagen molecule that can be acted upon by the enzyme lysyl oxidase to generate a maximum of four cross-links with other collagen molecules (*20*, *21*).

Accumulation of fluorescent advanced nonenzymatic glycosylation products in aortic connective tissue from untreated diabetic rats was 5.5 times greater than that in aortic tissue from untreated normal rats (Table 1). In contrast, the level in aortic tissue from aminoguanidine-treated diabetic rats was only 1.3 times that of untreated normal rats, despite exposure to identical levels of hyperglycemia for the same period of time. These data indicate that accumulation of advanced nonenzymatic glycosylation products is inhibited by aminoguanidine in vivo as well as in vitro.

The percentage of aortic connective tissue solubilized by each of the three specified procedures (acetic acid, CNBr, and pepsin) was significantly reduced in samples from untreated diabetic animals compared to those from normal animals (Table 1). Diabetic aortic connective tissue from untreated rats was 9 times more cross-linked than normal as assessed by acetic acid solubility, 2.8 times more as assessed by CNBr digestion, and 3.4 times as cross-linked according to pepsin digestibility. These data provide direct evidence that nonenzymatic mechanisms are involved in diabetes-induced increases in collagen cross-linking, since neither removal of lysyl oxidase–generated aldimine cross-links by acetic acid nor removal of lysyl oxidase–generated post-aldimine cross-links by pepsin affected the diabetes-induced increases in collagen cross-linking shown in Table 1.

In contrast, aortic connective tissue from aminoguanidine-treated diabetic animals was only 1.1 to 1.4 times more cross-linked than normal, despite exposure to identical levels of hyperglycemia for the same period of time. These data indicate that aminoguanidine prevents hyperglycemia-induced increases in nonenzymatic collagen cross-linking in vivo as well as in vitro. We cannot exclude the additional possibility that aminoguanidine may also interfere with lysyl oxidase–mediated collagen cross-link forma-

Table 1. Effect of aminoguanidine on formation of aortic fluorescent advanced nonenzymatic glycosylation products and aortic connective tissue cross-linking in diabetic and normal rats. Diabetes was induced in two of four experimental groups of male Lewis rats (three animals per group) by alloxan (40 mg/kg) administered intravenously and verified by plasma glucose determinations (≥250 mg/dl) prior to and at the termination of the experiment (*30*). Animals were killed by CO_2 inhalation, and the aortas were rapidly removed and placed on ice. Blood components were removed by washing with ice-cold buffer, and the samples were then finely minced with dissecting scissors. After delipidation with 5 ml of chloroform-methanol (2:1) as described previously (*14*), the insoluble pellets were washed five times with buffer and divided into aliquots for determination of protein cross-linking. After specific treatment, the soluble material was separated from insoluble material by centrifugation (50,000*g* for 1 hour), and all fractions were then lyophilized and hydrolyzed in 6*M* HCl for determination of total hydroxyproline (*31*). Solubility in 0.5*M* acetic acid was determined after 2 hours of extraction at 25°C and 24 hours at 4°C (*20*). Cyanogen bromide cleavage was performed as described in the legend to Fig. 3. Degradation by pepsin (Sigma) was carried out in 0.1*M* acetate buffer, *p*H 4.1, at a concentration of 1.0 mg/ml at 37°C for 48 hours (*32*). Data shown are mean ± SEM of three experiments.

Group	Advanced glycosylation products*	Percent collagen solubilized by		
		0.5*M* acetic acid	CNBr	Pepsin
		Untreated rats		
Normal	3.5 ± 0.1	18.0 ± 0.3	12.2 ± 0.1	50.9 ± 0.3
Diabetic	19.4 ± 0.8	2.0 ± 0.2	4.4 ± 0.1	15.0 ± 0.2
		Aminoguanidine-treated rats†		
Normal	2.8 ± 0.1	18.7 ± 0.3	13.6 ± 0.3	52.2 ± 0.2
Diabetic	4.5 ± 0.1	12.6 ± 0.3	10.6 ± 0.1	46.1 ± 0.4

*Specific fluorescence per microgram of hydroxyproline. †Each rat received 25 mg of aminoguanidine per kilogram of body weight per day.

tion, although the observed absence of any lathyrogenic effect in the aortic tissue from aminoguanidine-treated normal rats in these experiments (Table 1) makes a significant effect of this type unlikely.

With the use of aminoguanidine it should now be possible to evaluate the role played by extensive glycosylation-induced protein cross-linking in the development of chronic diabetic complications and the sequelae of aging. The potential ability of aminoguanidine or related compounds to prevent reduced arterial elasticity, increased peripheral vascular resistance, arteriosclerosis, and capillary basement membrane thickening in diabetes and aging is an exciting clinical possibility that needs further investigation.

REFERENCES AND NOTES

1. *Principles of Mammalian Aging*, R. R. Kohn, Ed. (Prentice-Hall, Englewood Cliffs, NJ, 1978).
2. D. E. Harrison and J. R. Archer, *Exp. Gerontol.* **13**, 75 (1978).
3. S. L. Schnider and R. R. Kohn, *J. Clin. Invest.* **66**, 1179 (1980).
4. ———, *ibid.* **67**, 1630 (1981).
5. V. Monnier and A. Cerami, *Science* **211**, 491 (1981).
6. M. Brownlee, H. Vlassara, A. Cerami, *J. Exp. Med.* **158**, 1739 (1983).
7. A. S. Eble, S. P. Thorpe, J. W. Baynes, *J. Biol. Chem.* **258**, 9506 (1983).
8. R. R. Kohn, A. Cerami, V. Monnier, *Diabetes* **33**, 57 (1984).
9. S. Pongor, P. C. Ulrich, F. A. Bencsath, A. Cerami, *Proc. Natl. Acad. Sci. U.S.A.* **81**, 2684 (1984).
10. M. J. C. Kent, N. D. Light, A. J. Bailey, *Biochem. J.* **225**, 745 (1985).
11. J. W. Baynes *et al.*, in *Methods in Enzymology: Posttranslational Modifications*, F. Wold and K. Moldave, Eds. (Academic Press, New York, 1984), vol. 6, pp. 88–98.
12. H. B. Mortensen and C. Christophersen, *Clin. Chim. Acta* **134**, 317 (1983).
13. M. Brownlee, H. Vlassara, A. Cerami, in *Diabetic Complications: Scientific and Clinical Aspects*, M. J. C. Crabbe, Ed. (Pitman, London, 1986).
14. V. Monnier, R. R. Kohn, A. Cerami, *Proc. Natl. Acad. Sci. U.S.A.* **81**, 583 (1984).
15. P. A. S. Smith, *Derivatives of Hydrazine and Other Hydronitrogens Having N-N Bonds* (Benjamin/Cummings, Reading, MA, 1983).
16. S. Patai, Ed., *The Chemistry of the Hydrazo, Azo, and Azoxy Groups* (Wiley, London, 1975).
17. J. Higgins and H. F. Bunn, *J. Biol. Chem.* **256**, 5204 (1981).
18. F. S. Heinemann and J. Ozols, *ibid.* **259**, 791 (1984).
19. H. Rosenberg, J. B. Modrak, J. M. Hassing, W. A. Al-Turk, S. J. Stohs, *Biochem. Biophys. Res. Commun.* **91**, 498 (1979).
20. E. J. Miller and R. K. Rhodes, *Methods Enzymol.* **82A**, 33 (1982).
21. E. J. Miller and S. Gay, *ibid.*, p. 1.
22. M. Bradford, *Anal. Biochem.* **72**, 248 (1976).
23. P. M. Gallop and S. Seifter, *Methods Enzymol.* **6**, 635 (1963).
24. G. Chandrakasan, D. A. Torchia, K. A. Piez, *J. Biol. Chem.* **251**, 6062 (1976).
25. B. Broadsky and E. F. Eikenberry, *Methods Enzymol.* **82A**, 127 (1982).
26. J. A. Last, J. Baer, C. Millson, *Connect. Tissue Res.* **4**, 149 (1976).
27. A. H. Gordon, in *Laboratory Techniques in Biochemistry and Molecular Biology*, T. S. Work and E. Work, Eds. (North-Holland, Amsterdam, 1975), vol. 1, part 1.
28. A. H. Reisner, P. Nemes, C. Bucholtz, *Anal. Biochem.* **64**, 509 (1975).
29. C. R. Merrill, R. C. Switzer, M. L. Van Keuren, *ibid.* **105**, 361 (1980).
30. A. A. Rossini, M. A. Arcangeli, G. F. Cahill, Jr., *Diabetes* **24**, 516 (1975).
31. H. Stegemann and K. Stadler, *Clin. Chim. Acta* **18**, 267 (1967).
32. A. P. Ryle, *Methods Enzymol.* **19**, 316 (1970).

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High Resolution of Mouse Chromosomes: Banding Conservation Between Man and Mouse

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A detailed schematic representation of high-resolution G-banding patterns was prepared from elongated and finely banded mitotic chromosomes of the mouse. Such chromosomes can be obtained from both animal tissue and cell lines by a simple protocol, facilitating precise demarcation of breakpoints in chromosome rearrangements and aiding in the sublocalization of genes. Regions of subbanding homology were observed between human and mouse chromosomal segments known to have conserved gene assignments, an indication that, at the cytogenetic level, extensive regions of the mammalian genome may remain intact after 60 million years of species divergence.

CHROMOSOME BANDING PROCEDURES allow identification of individual mammalian chromosomes and, when combined with appropriate gene-mapping strategies, provide information on the location of genes. Recently, the analysis of human chromosome aberrations and comparative cytogenetics of humans and primates have benefited from high-resolution banding techniques (*1*). High-resolution cytogenetics provides precision in the delineation of chromosomal breakpoints and assignment of gene loci, greater than with earlier techniques, since analysis of late prophase subbanding reveals more than twice the number of bands seen at metaphase. Subbanding analysis of chromosomes has also become important in the study of the structural organization of chromosomes, with chromosomal DNA segments as small as about 1200 kilobases now being visualized at the light microscopic level (*2*). In mapping studies, the sublocalization of structural genes (and even DNA sequences whose function is not yet known) can be precisely established by in situ molecular hybridization of cloned DNA probes to high-resolution mitotic chromosomes (*3*).

In the past, the mouse has been a preferred mammalian species for in vivo genetic analysis, and numerous mouse cell lines have been established for the study of mutagenesis, gene regulation in vitro, and somatic cell genetics. Here we describe the use of actinomycin D in combination with acridine orange to induce excellent high-resolution mouse chromosome subbanding. Treatment with actinomycin D for 1 hour before the cell harvest and fixation procedure yields highly elongated chromosomes with an adequate number of mitoses for analysis (*4*). Acridine orange, when added for 20 minutes at the hypotonic step in the cell harvest procedure, holds chromosomes in an extended state, apparently by intercalating between DNA base pairs (*5*). We believe such pretreatment also induces more sharply defined chromosome subbanding.

Mouse chromosomes for high-resolution analysis were prepared from splenic lymphocytes of DBA/2 mice. Spleens were dispersed with a tissue screen, and the single-cell suspension (1×10^6 to 2×10^6 cells) was cultured in 10 ml of RPMI 1640 medium supplemented with 20% fetal calf serum, 2% L-glutamine, penicillin (50 U/ml), and streptomycin (50 μg/ml). Purified phytohemagglutinin (Burroughs Wellcome HA16, 0.3 μg/ml of cell culture) was then added and cultures were incubated at 37°C (*6*). After 72 hours, actinomycin D (3.0 μg/ml) and Colcemid (0.025 μg/ml) were added and incubation was continued for an additional hour. The cells were then centrifuged at 800*g* and gently resuspended in 9 ml of 0.075*M* KCl containing acridine orange (10 μg/ml). The cell suspension was then incubated for an additional 20 minutes at 37°C; the cells were then fixed in 3:1 methanol: acetic acid. Fixed cell suspension was then dropped onto ethanol-cleaned, dry slides from a distance of about 30 cm.

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