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17 JULY 1992
VOL. 257 • PAGES 297-452

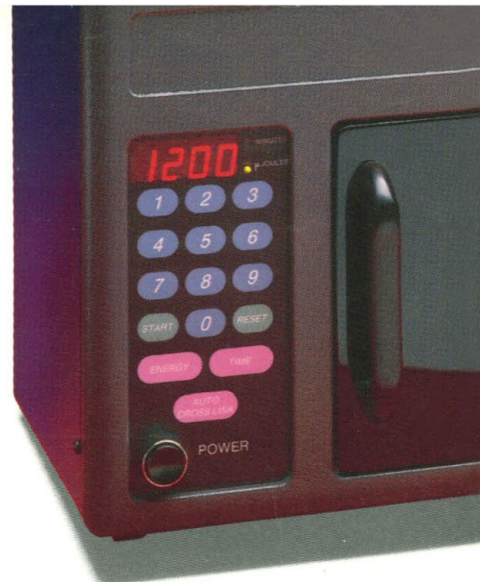
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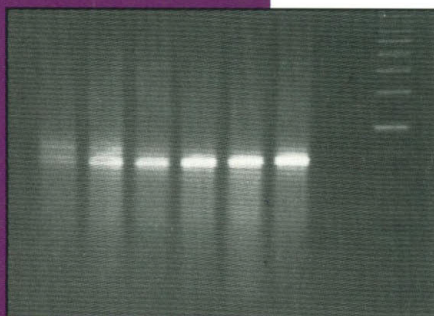


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- Reduce PCR™ False Positives
- UV Crosslink DNA or RNA to Hybridization Membranes
- Fast Results



Reduce PCR False Positives:

Contaminating DNA can be rendered inactive as a template for PCR by treatment in the Stratalinker® UV crosslinker. Reagents suspected of being contaminated can be pre-irradiated with 254 nm light (either in measured energy doses or by using the timer option) to prevent extraneous sequences from being amplified, which may result in false positives. Because of its simplicity and speed, pretreatment with the Stratalinker® UV crosslinker can be easily integrated into PCR protocols as a precautionary measure.

Six identical tubes, each containing 100 ng of contaminating DNA (4.8 kb linear fragment) in reaction buffer were irradiated at successively higher energy levels in the Stratalinker UV crosslinker. Following treatment, 100 ng of control DNA (4.1 kb linear fragment) was added to each tube, along with recommended amounts of primers, nucleotides, and polymerase. PCR was performed according to the GeneAmp™ kit (Perkin-Elmer Cetus Instruments) instructions. Amplification of the contaminating DNA yields a 1.8 kb fragment, whereas the control DNA with the same primers yields a 1.1 kb fragment. Lanes 1-6: 0, 25, 50, 100, 200, and 400 mJ/cm² UV energy, respectively. Lane 7: Stratagene's Kb size ladder (500 ng).

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UV Crosslink DNA or RNA to Membranes:

The degree of ultraviolet crosslinking can dramatically affect the binding and sensitivity of nucleic acid hybridization (see accompanying chart). The versatile Stratalinker® UV crosslinker can be used for the following applications:

- Binding RNA or DNA to nitrocellulose, nylon or hybrid membranes for Northern, Southern, dot, or slot blot analysis (1, 2).
- Linking DNA to filters for bacterial or phage library screening.
- DNA nicking in agarose gels prior to blotting (3).
- Dimer formation to perform partial digests for rapid gene mapping (4).
- Confirming recA+ versus recA- genotypes in *E.coli* strains through UV sensitivity testing (5).

1. Khandjian, E.W. *Biotechnology* 5 February 1987.
2. Church, G.M., Gilbert, W. *P.N.A.S.* 81: 1991-1995, 1984.
3. Vollrath, D. and Davis, R.W. *Nucl.Acids Res.* 15:7865-7876, 1987.
4. Whittaker, P.A., Southern E.M. *Gene* 41: 129-134, 1986.
5. Maniatis, T., et al *Molecular Cloning: A Laboratory Manual*. Cold Spring Harbor Laboratory.



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* patents pending

**The polymerase chain reaction (PCR) process is covered by patents issued to Cetus Corporation.
† GeneAmp is a registered trademark of Cetus Corporation



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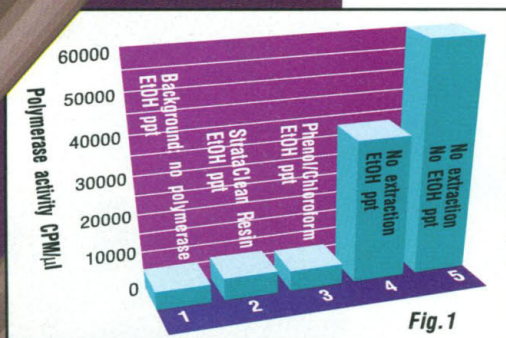


Fig. 1

Remove Polymerase Activity after PCR with StrataClean Resin

StrataClean[™] resin has proven to be a safe, fast, and efficient method for the removal of polymerase activity from PCR reaction mixtures. Using StrataClean resin, it is also possible to eliminate an ethanol precipitation step before cloning PCR amplified DNA.

Figure 1. Comparison of StrataClean resin and phenol/chloroform extraction methods for removal of Polymerase activity following amplification reactions. Four identical 100μl amplification reactions were treated as follows: no extraction; two extractions with StrataClean resin; two extractions with phenol/chloroform; and a single precipitation with ethanol. The samples were then assayed for polymerase activity at 72°C using a modified activated calf thymus/gap filling assay, essentially as described by Maki, *et. al.* (2). Background activity was measured by assaying an amplification reaction without any added polymerase.



Fig. 2

Sequence Quality Plasmid Templates from Mini-preps Using StrataClean Resin

StrataClean resin has also been shown to produce sequencing quality plasmid templates when used in either a rapid boiling mini-prep or as a substitute for phenol in a standard alkaline lysis mini-prep. The key to the rapid mini-prep is that no precipitation step is required. StrataClean resin is used to remove the unwanted cellular protein quickly and efficiently.

Figure 2. Sequence derived from DNA purified using StrataClean resin with both the standard rapid boiling mini-prep procedure and the alkaline lysis mini-prep procedure. Panel A: StrataClean resin rapid boiling mini-prep; Panel B: phenol/chloroform alkaline lysis mini-prep; Panel C: StrataClean resin alkaline lysis mini-prep.

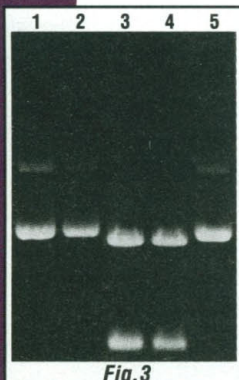


Fig. 3

Extract Restriction Endonucleases and many DNA Modifying Enzymes from Nucleic Acids

Quantitative removal of restriction enzymes from DNA can be accomplished in a matter of minutes with StrataClean resin and eliminates the hazards associated with liquid phenol extractions. The StrataClean resin extraction relies on the use of patented hydroxylated silica particles which exhibit characteristics similar to phenol^{1,2}. It is supplied as a 25% slurry which is non-toxic, non-flammable and odor-free. StrataClean resin has a very high affinity for proteins and a very low affinity for nucleic acids at neutral pH (>1900:1 respectively).

1. U.S. Patent Serial No. 4,923,978 2. Strategies Vol. 3 Number 4

Figure 3. Ethidium stained agarose gel. Lane 1: control uncut plasmid DNA, Lane 2: the same DNA after standard StrataClean resin extraction, Lane 3: plasmid DNA digested with 4 units *Pvu* II, Lane 4: plasmid DNA after standard StrataClean resin extraction then digested with *Pvu* II, Lane 5: 24 units *Pvu* II extracted with StrataClean resin from 20 microliters of 1X Universal buffer, plasmid DNA then added and incubated at 37°C for 18 hours.

* The polymerase chain reaction (PCR) process is covered by patents issued to Cetus Corporation.



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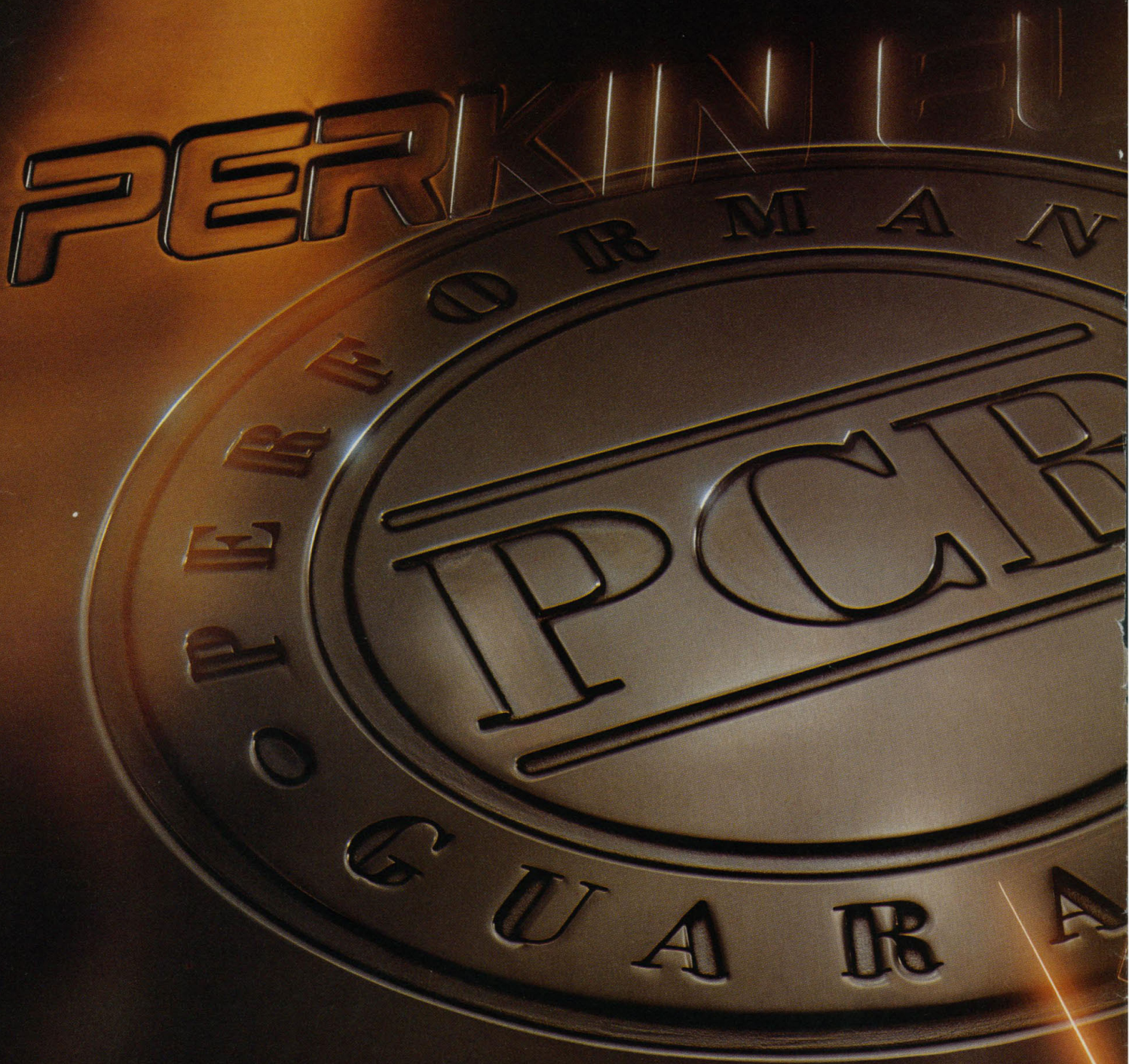
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Color-enhanced image of a familiar formula written with an atomic force microscope. The lines of the letters are about 10 nanometers wide and 5 nanometers deep. The patterns are machined into a soft molybdenum oxide layer that was grown on a molybdenum sulfide

substrate. See page 375. New techniques and instruments, especially in biomedical research, are the focus of the Science Innovations '92 meeting (21 to 25 July in San Francisco) sponsored by AAAS and *Science*; see related news stories on page 344. [Photo: Charles M. Lieber]



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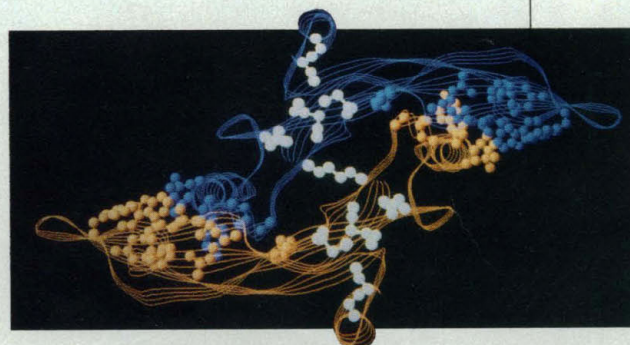
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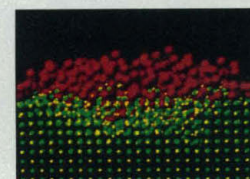
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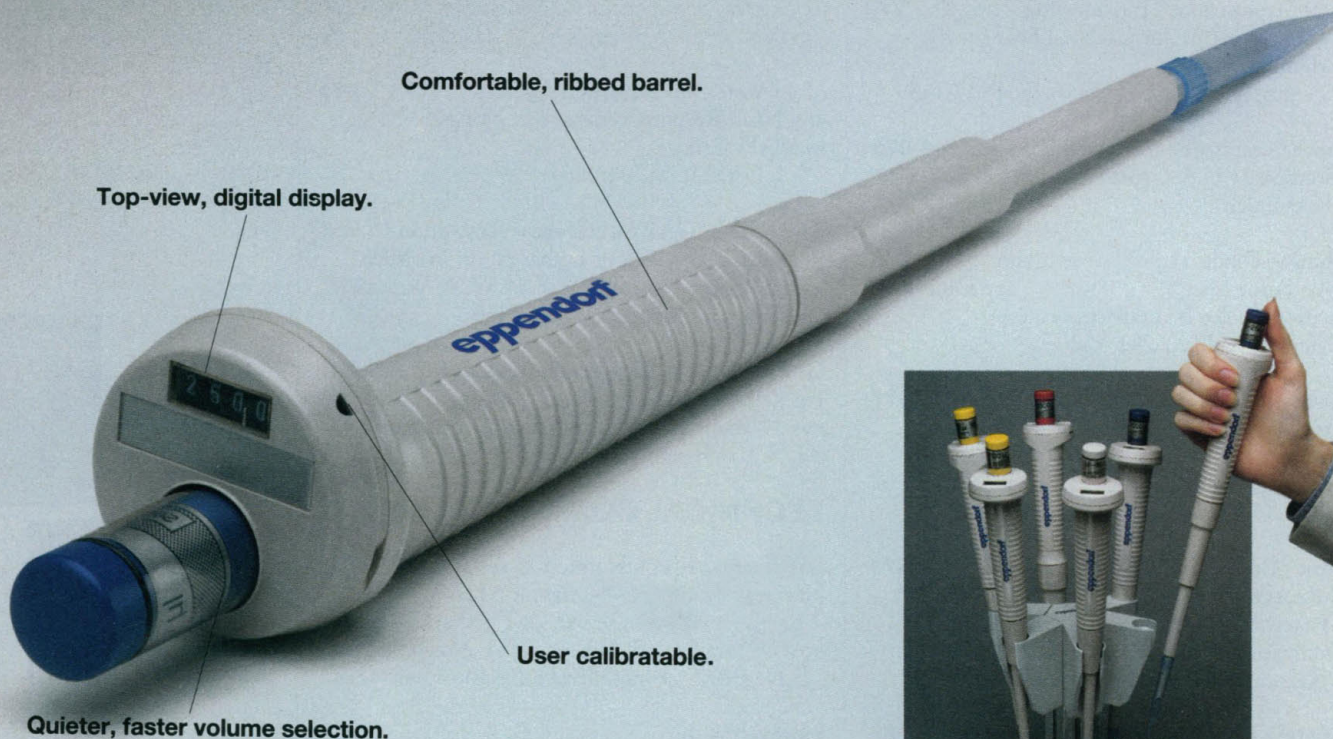
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Precambrian predation

Animals first developed mineralized skeletons about 550 million years ago as part of a major evolutionary radiation known as the Cambrian explosion. The causes leading to this explosion are under debate; one hypothesis is that it was to escape predation. Bengtson and Zhao (p. 367) report fossil evidence supporting this notion. They report that about 3 percent of well-preserved fossils of the earliest known animals with mineralized skeletons (the late Precambrian *Cloudina*) have round holes, 40 to 400 micrometers in diameter, that are characteristic of attack by predators. Larger holes tend to occur in larger fossils; this observation suggests that the predator was selecting the prey for size.

Warm waters

Causes of recent sea level changes include continued slow rebound of the crust following deglaciation, melting of glaciers and ice sheets, and thermal expansion of the oceans following recent warming. Roemmich (p. 373) used hydrographic data collected during the past 42 years off the coast of southern California to evaluate the effect of thermal expansion. The data show that the upper 100 meters of the water column has warmed by 0.8°C, which resulted in a sea level rise of 0.9 millimeter per year, a value consistent with coastal tide gauge records.

Hemophilia and puberty

Patients with Hemophilia B Leyden, an inherited disorder linked to the X chromosome, usually improve after the onset of puberty because testosterone,

Clusters colliding with surfaces

Laboratory experiments that produce conditions of high temperature and density could open up new chemical reaction pathways. Cleveland and Landman (p. 355) report on theoretical investigations into the dynamics of energetic collisions between solid surfaces and atomic clusters. They used molecular dynamics simulations to study the impact of large argon clusters (561 atoms) on sodium chloride surfaces. A "piling-up" of the shock wave and inertial confinement of material led to extreme conditions in the impact region. Reactants could be embedded in the clusters to catalyze reactions during the collision.

an androgenic hormone, can promote transcription of the gene for the blood clotting factor IX. Crossley *et al.* (p. 377) found a form of the disease in which there is no improvement after puberty. In this form, mutations in the promoter region of the factor IX gene at positions -20 and -26 disrupted binding of a liver-enriched transcription factor, LF-A1/HNF4. Patients who recover also have mutations at -20 but no mutations at -26. The mutation at -26, however, is within an overlapping androgen-responsive element (ARE). If the ARE is intact (no mutation at -26), its activation by testosterone can direct transcription of factor IX even though the overlapping LF-A1/HNF4 site is inactivated by the mutation at -20.

Dendritic cells as HIV-1 reservoirs

Infection with human immunodeficiency virus type-1 depletes the population of CD4⁺ T cells in the human body, thus opening the door to opportunistic infections, but very few CD4⁺ cells are infected with HIV-1 at any one time. Cameron *et al.* (p. 383) show that dendritic cells, a type of antigen-presenting cell that can stimulate the T cell response, can transfer the virus to

CD4⁺ cells that have been activated by superantigens. The dendritic cells carry HIV-1 but are not actively infected (the virus is not making copies). These cells form clusters with CD4⁺ cells for antigen presentation; the T cells, which replicate in this process, are in the form most susceptible for infection. After cluster formation with the dendritic cells, the previously uninfected CD4⁺ cells replicate virus and die after viral release.

NO effect

Nitric oxide (NO) has a number of physiological roles, including helping macrophages kill cells, acting as a neurotransmitter, and participating in vasodilation. Two reports discuss the mediatory role of NO and the enzyme that produces NO, NO synthase (NOS). A side effect of cardiac surgery is stunning, a depression of myocardial contractility. This effect may be caused by pro-inflammatory cytokines, polypeptides that are secreted by various cell types in response to injury. Finkel *et al.* (p. 387) show that inhibition of NOS can block the contractility effects of cytokines such as interleukin-2. Burnett *et al.* (p. 401) demonstrate that NO can mediate the penile erection response in rats. They found high levels of NOS activity in

neurons that innervated blood vessels and the corpora cavernosa of the penis.

Functional N-type calcium channels

Excitable cells can release neurotransmitters in response to influxes of extracellular calcium, a process that is often mediated by N-type calcium channels. This family of voltage-sensitive channels, which are blocked by ω -conotoxin, are multisubunit proteins; although different subunits have been cloned and expressed, it has proved difficult to reconstitute a fully functional N-type calcium channel. Williams *et al.* (p. 389) report the sequence of a human neuronal α_1 subunit, α_{1B} , that when coexpressed with the human neuronal α_{2b} and β_2 subunits, form voltage-sensitive calcium channels with high-affinity ω -conotoxin binding sites.

Neuron survival

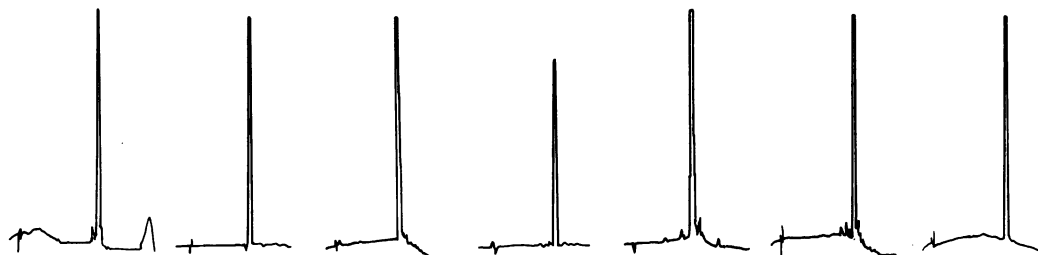
Mature vertebrate sympathetic neurons require neurotrophic factors such as nerve growth factor (NGF) for their survival, but the precursor cells that become sympathetic neurons do not require NGF; in fact, they are unresponsive to NGF and lack NGF receptors. Birren *et al.* (p. 395), working with an immortalized cell line of progenitor cells to sympathetic neurons, find that electrical activity (membrane depolarization) induces the expression of the signal-transducing part of the NGF receptor, p140^{trk}. Depolarization did not induce expression of the low-affinity NGF receptor p75. The cells expressing p140^{trk} become responsive to NGF as well as fibroblast growth factor and developed into neurons.

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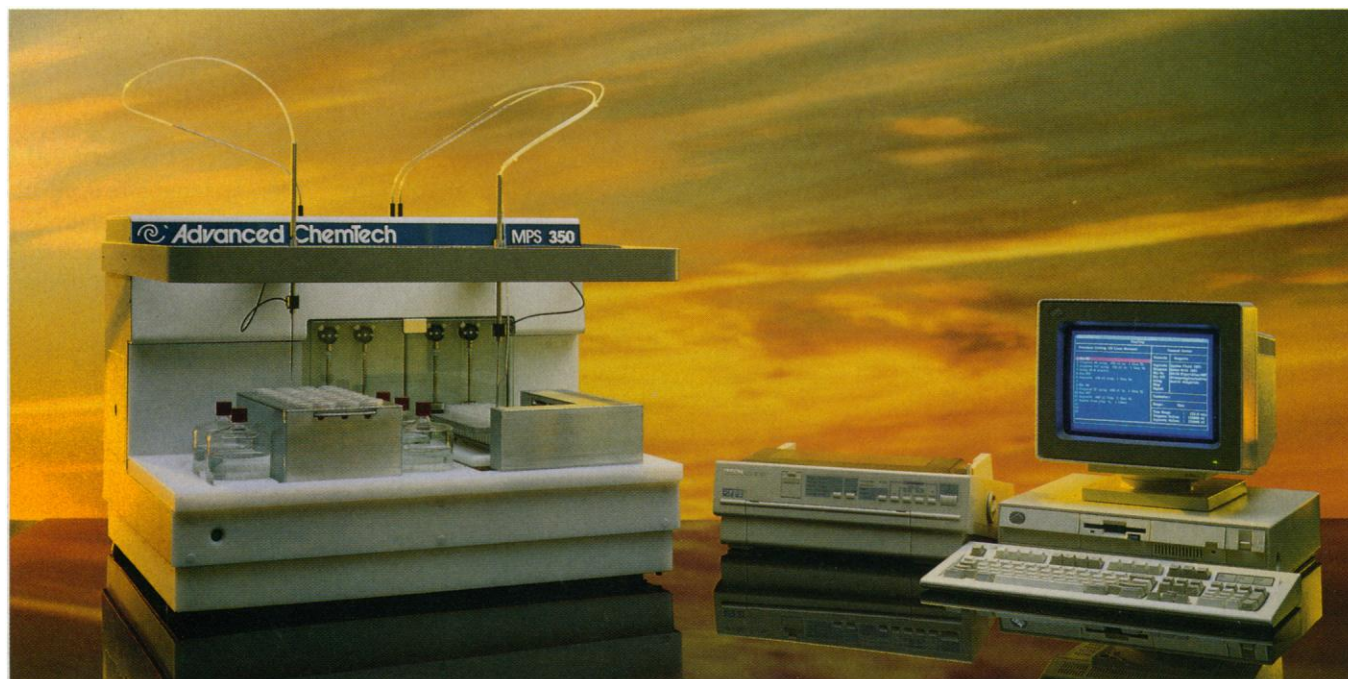
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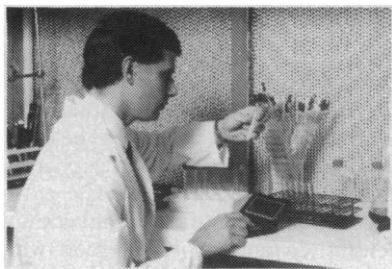
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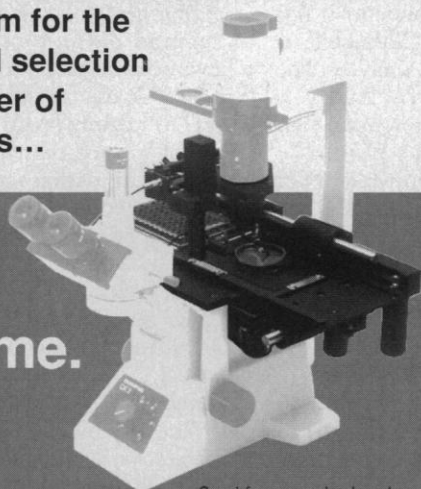
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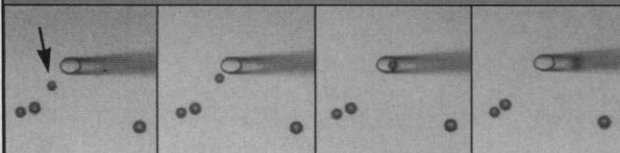
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A Policy Forum
Reprinted from *Science*

MARTIN B. HOCKING



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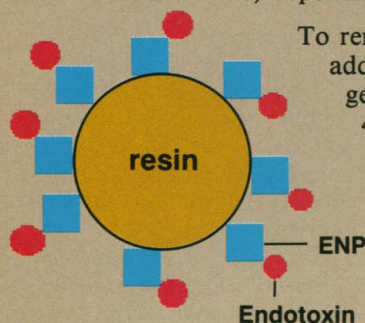
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References

1. Alpert, G., G. Baldwin, C. Thompson, N. Wainwright, T. J. Novitsky, Z. Gillis, G. R. Siber, J. Parsonnet, and G. R. Fleisher. 1992. Limulus antilipopolysaccharide factor protects rabbits from meningococcal endotoxin shock. *J. Infect. Dis.* 165:494-500.
2. Wainwright, N. R., R. J. Miller, E. Paus, T. J. Novitsky, M. A. Fletcher, T. M. McKenna, and T. Williams. 1990. Endotoxin binding and neutralizing activity by a protein from *Limulus polyphemus*. p. 315-325. In A. Nowotny, J. J. Spitzer, and E. J. Ziegler, (ed.) Cellular and molecular aspects of endotoxin reactions. Elsevier Science Publishers B.V., Amsterdam.

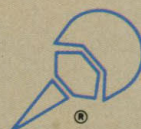
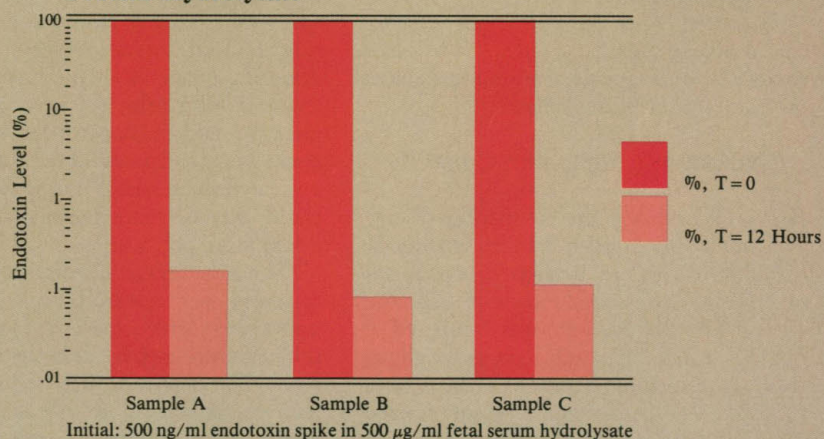
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Recombinant Hemoglobin 250 mg/ml	200 ng/ml	0.1 ng/ml	> 99.9
Vaccine (Chicken) 2.5 mg/ml	1000 ng/ml	90.0 ng/ml	91.0**
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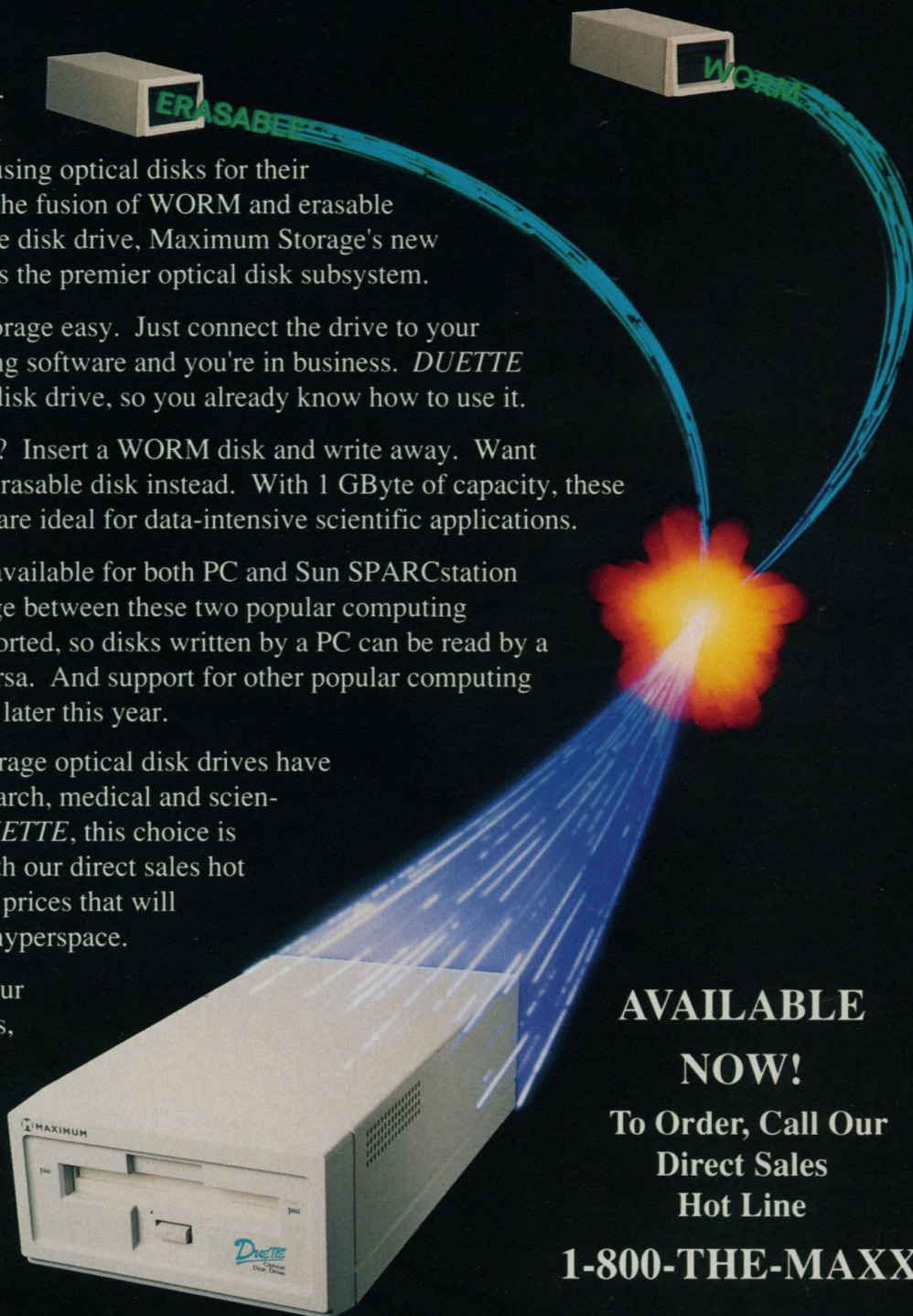
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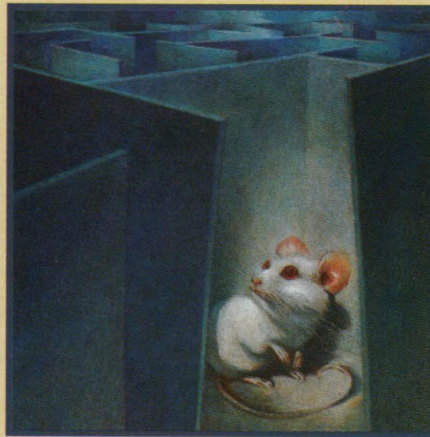
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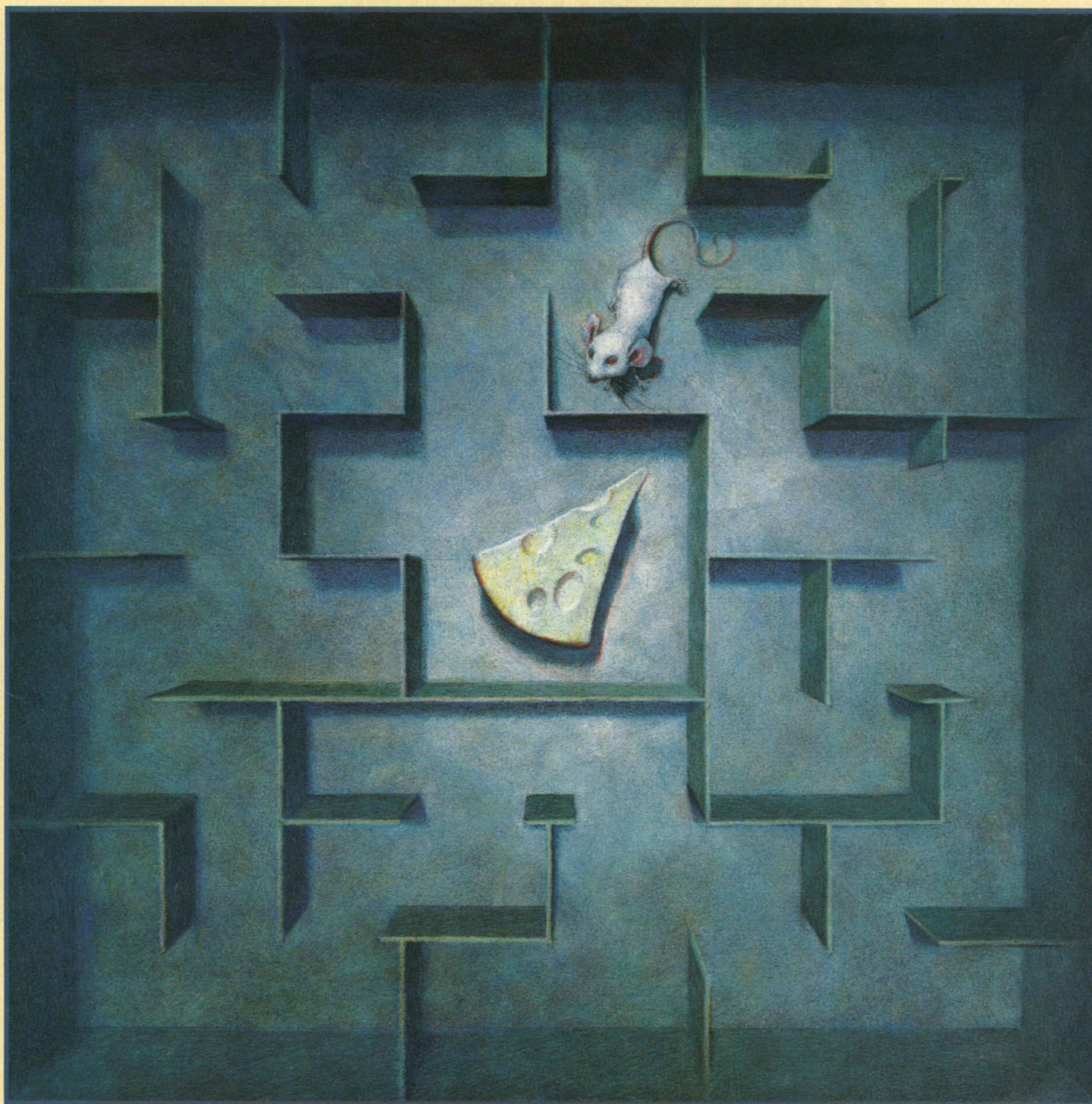
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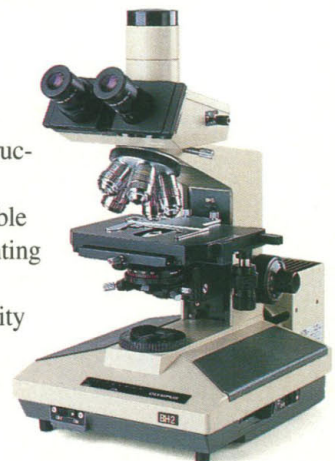
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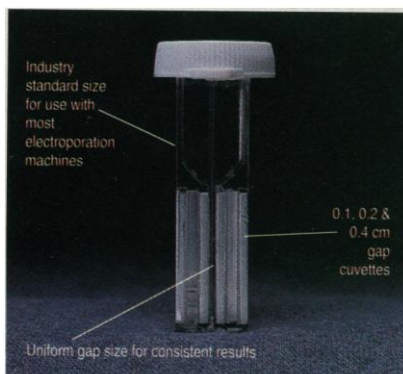
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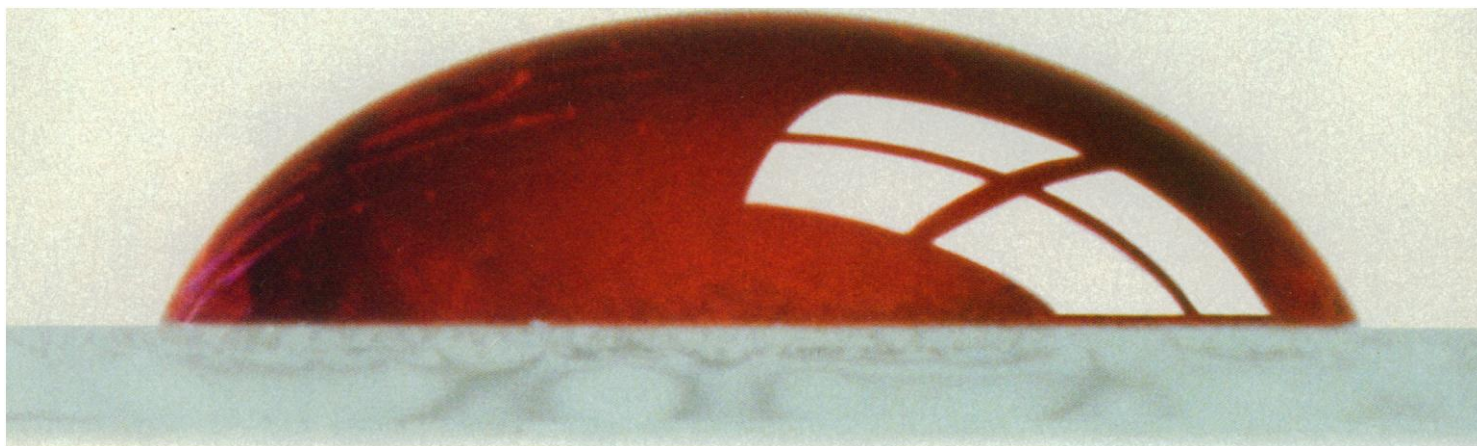
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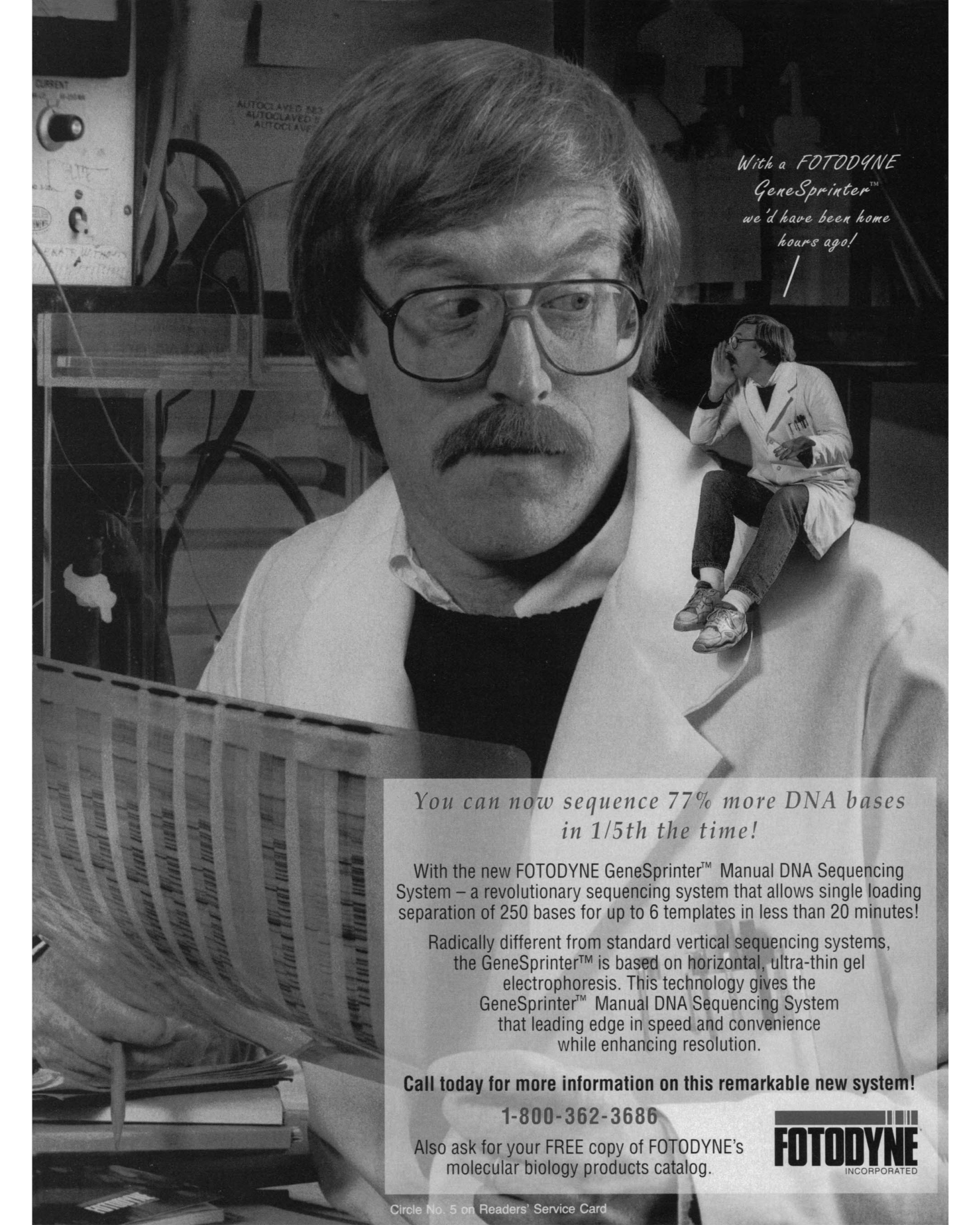

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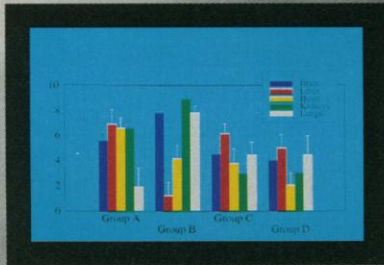
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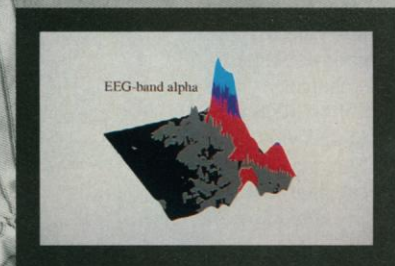
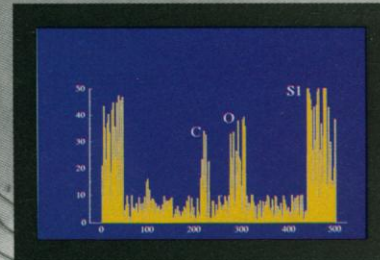
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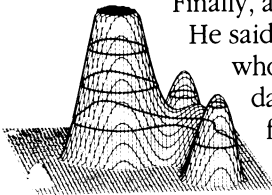
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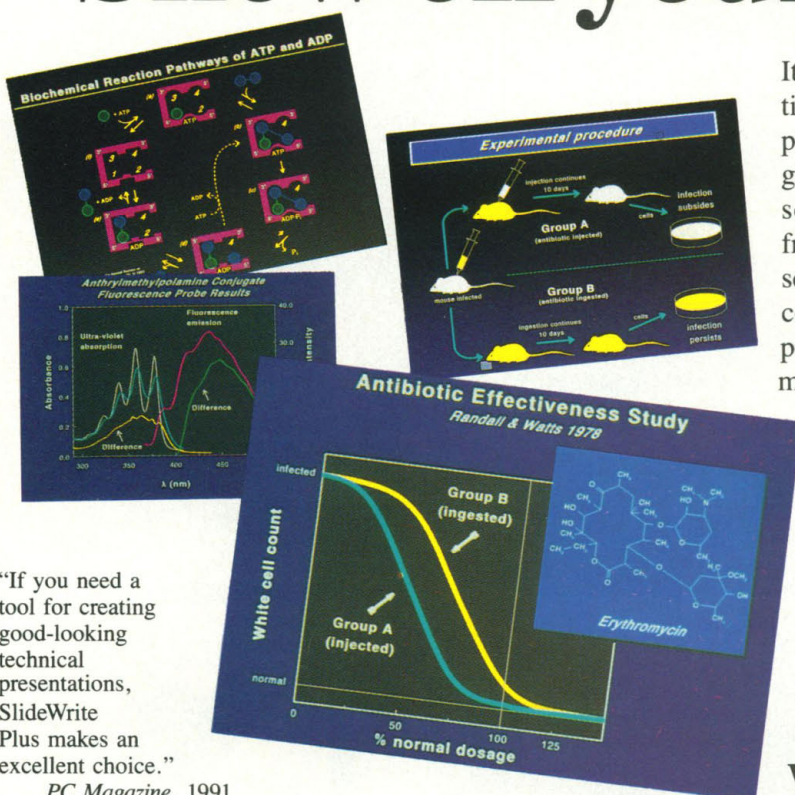
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Drug Prohibition in the United States: Costs, Consequences, and Alternatives

An Article Reprinted from *Science* (1 September 1989)

ETHAN A. NADELMANN

FROM THE ABSTRACT:

"Drug legalization" increasingly merits serious consideration as both an analytical model and a policy option for addressing the "drug problem." Criminal justice approaches to the drug problem have proven limited in their capacity to curtail drug abuse. They also have proven increasingly costly and counterproductive. Drug legalization policies that are wisely implemented can minimize the risks of legalization, dramatically reduce the costs of current policies, and directly address the problems of drug abuse. Twelve pages.



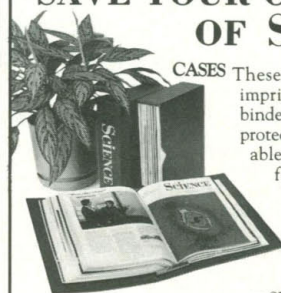
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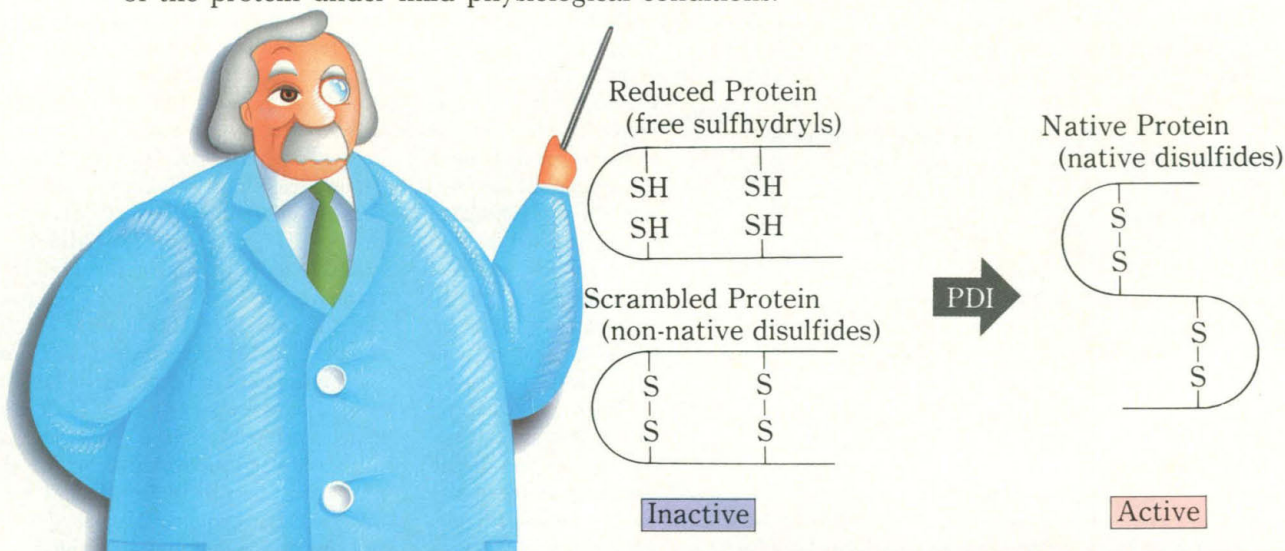
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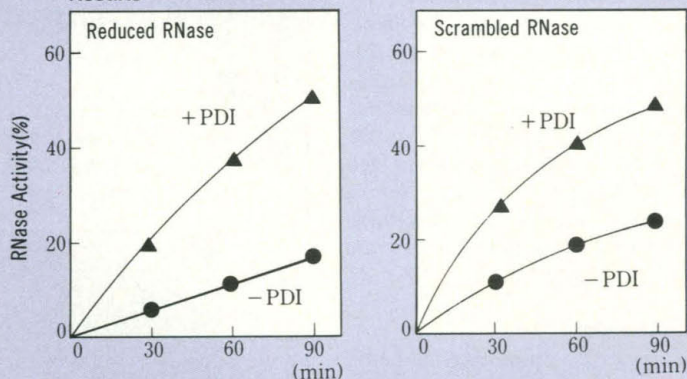
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	Final conc.
Substrate protein	200 μ g/ml
Sodium phosphate buffer, pH 7.5	100mM
Glutathione	0.2mM
Reduced glutathione	2mM
PDI	20 μ g/ml

Results



Code No.7318:1mg/vial



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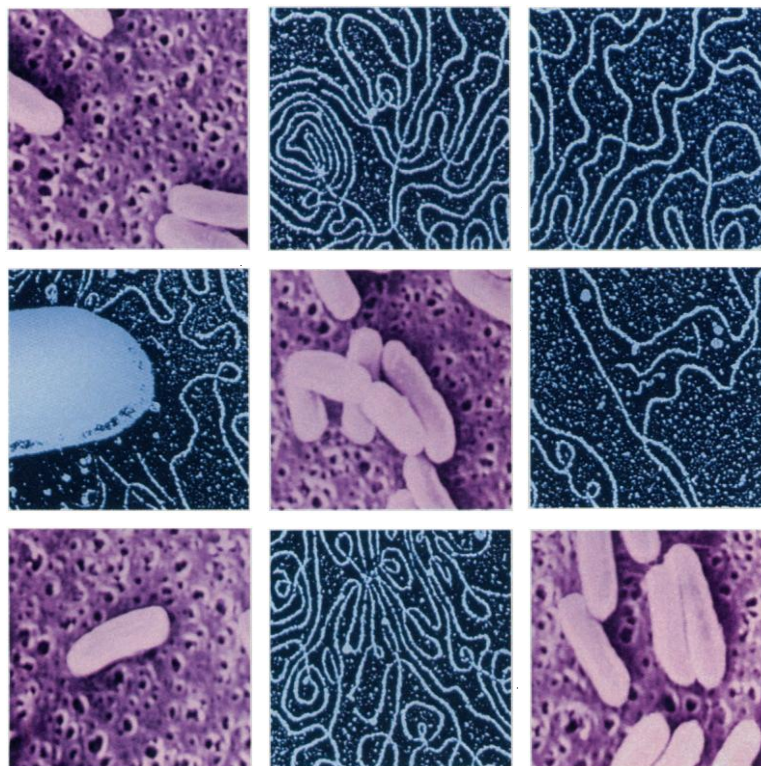
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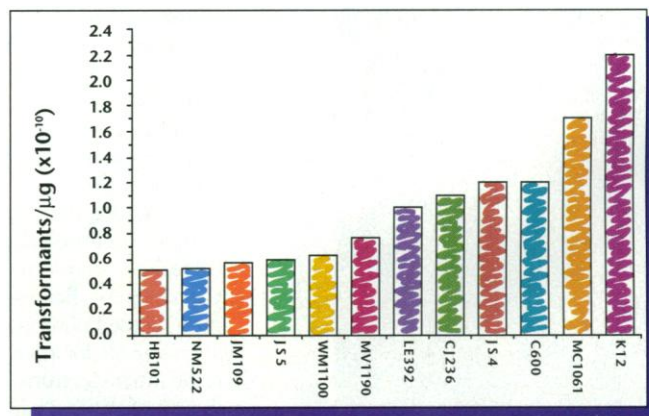
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The Hormonal Control of Gene Transcription

edited by P. Cohen and J.G. Foulkes

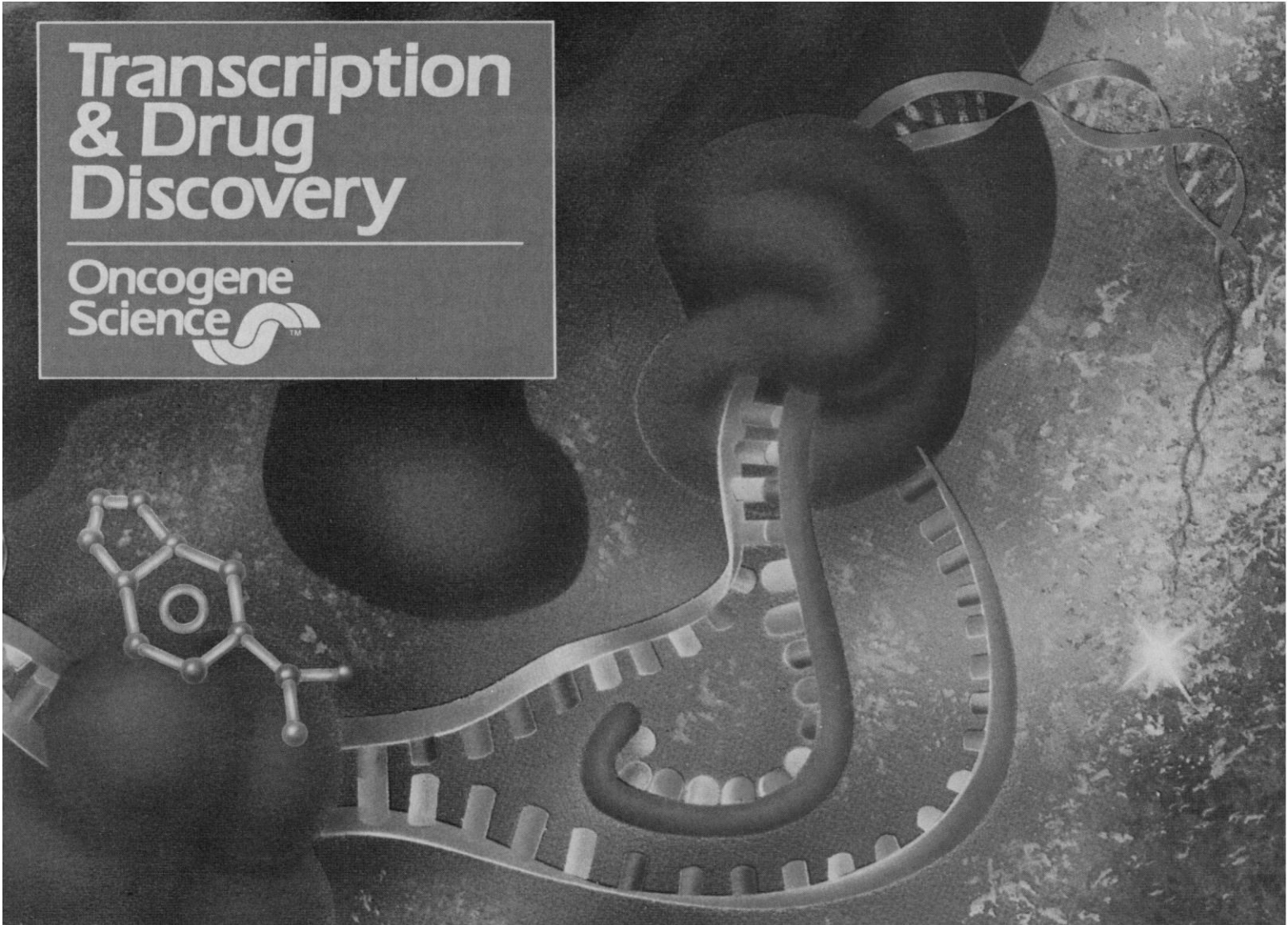
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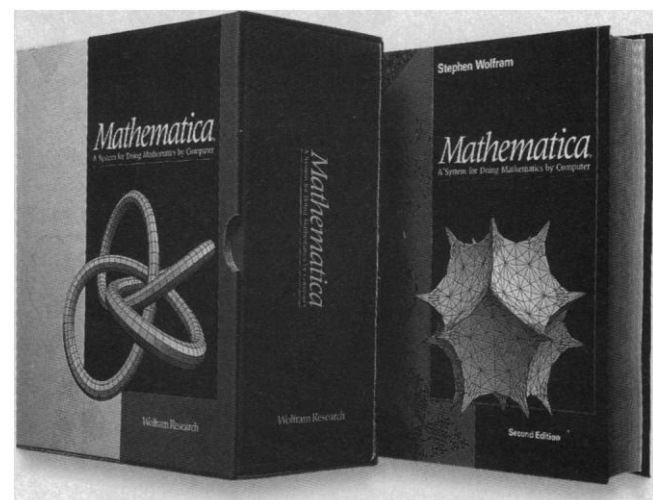
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