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15 JUNE 1990

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**FRONTIERS IN BIOTECHNOLOGY**  
**Tolerance in the Immune System**



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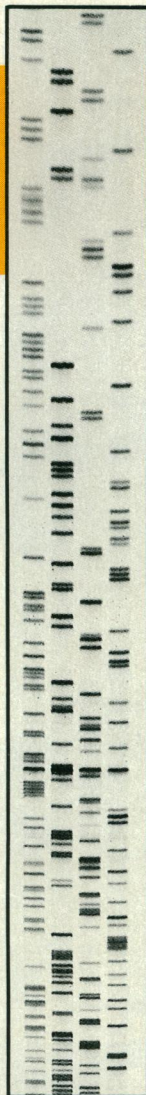
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1. Nielson, K. and Mathur, E.J. (1990) Strategies 3:17-19.
2. Nielson, K. and Mathur, E.J. Manuscript in preparation.

3. Nielson, K. and Mathur E.J. (1989) U.S. patents filed.
4. Mullis, K.B., and Faloona, F.A. (1987) Meth. Enzymol. 155:335-350.

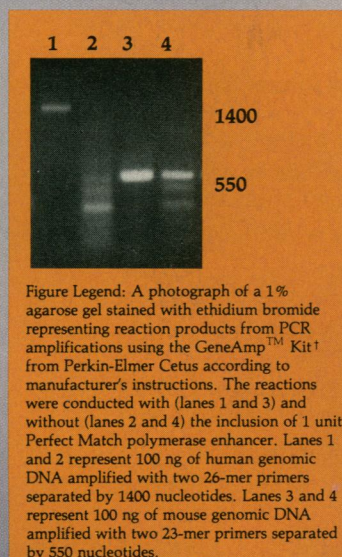


Figure 1 shows two examples of *in vitro* amplification reactions that are significantly enhanced by the addition of Perfect Match polymerase enhancer to the polymerase preparation. Note that in lanes 1 and 2, the desired PCR product cannot be detected unless Perfect Match polymerase enhancer is added to the amplification reaction.

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**COVER** An allegory of immune tolerance, inspired by M. C. Escher. Immune function depends on distinguishing between self and nonself. When the immune system cannot determine whether an antigen is self or foreign, self tolerance may break down and autoimmune disease ensues. The articles on tolerance in the immune system begin on page 1335. [Concept by Linda J. Miller, illustration by Julie Cherry]

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## This Week in SCIENCE

### Tolerance in the immune system

**T**HROUGHOUT the life of each individual, self and foreign materials are presented to the immune system; the system then responds with tolerance or immunity. Not infrequently the ability of the immune system to appropriately discriminate what is self from what is foreign goes awry; then tumors, autoimmunity, and rampant infections may ensue. Both in the thymus during perinatal life and in the peripheral lymphoid organs throughout life, cells of the immune system are activated, repressed, or remain silent following exposure to immunogenic and tolerogenic materials. Recent insights into the induction of immune tolerance under normal conditions, in disease, and in association with transplantation are the subjects of this special issue (pages 1273 and 1335 to 1393).

### The path less traveled

**L**IGHT is converted to chemical energy in bacteria by a membrane protein called the photosynthetic reaction center. Although two possible pathways exist for charge separation and the transfer of electrons in the reaction center, only one is used. The pathways are not identical but homologous and are thought to have diverged from a single ancestral path; divergence may have helped to maximize the efficiency of the conversion reaction. In a series of genetic experiments, Robles *et al.* have examined how protein function is affected by the exchange of homologous helical subunits between the two pathways (page 1402); these exchanges produced reaction centers with partial symmetry that probably were somewhat like the proposed homodimeric ancestral molecule. Symmetrization derailed electron transfer, but, in some cases, following point mutations, transfer activity was restored. The studies have identified some structural elements of the protein that are critical for function and some biophysical features of photosynthesis.

### Guppy love

**W**HEN it comes to choosing a mate, female guppies from populations in which males are brightly colored have a strong preference for conspicuously pigmented males; females from populations with dull males pay less attention to color in selecting their mate (page 1405). Guppies from seven separate populations that had been collected from six streams in Trinidad were evaluated and compared; the orange coloration on the male bodies covered from 5 to 17% of total body area. Males and females were raised separately, but, when they were brought together, the strong color preferences of the females in populations with bright males were expressed. Houde and Endler note that as early as the 1870s, Charles Darwin had suggested that certain male sexual-display traits (of which color pattern is an example) might be evolving in parallel with female mating preferences. The reproductive isolation that this would foster would then tend to promote further speciation and divergence.

### Halting vascularization

**T**HE production of new blood vessels in various tissues of the body—angiogenesis—is a continuing and normal process; in addition, angiogenesis can contribute to the pathology of various diseases such as rheumatoid arthritis and malignancies. It is therefore expected that methods (either stimulatory or inhibitory) for modulating angiogenesis will have a variety of clinical applications. Moses *et al.* have identified a cartilage-derived substance, CDI, that inhibits angiogenesis when tested in vivo and that also inhibits processes that are associated with angiogenesis—the proliferation and migration of capillary endothelial cells—in two in vitro bioassays (page 1408). That cartilage is the source of an inhibitor of angiogenesis is not surprising, because cartilage has long been known to be a tissue that naturally resists vascularization.

### Herpesvirus entry site

**A** major membrane portal through which the herpes simplex virus (HSV-1) can enter vertebrate cells is the fibroblast growth factor (FGF) receptor (page 1410). (FGF is a growth factor that plays a role in wound healing, angiogenesis, and arterial wall proliferation.) The viruses first attach to glycosaminoglycan molecules on the surface of a target cell; then, either through an integral or an adsorbed FGF molecule, the virus may bind to the FGF receptor and be internalized. Many cells normally have FGF receptors; Kaner *et al.* show that others that lack FGF receptors can be made to take up virus if receptors are inserted into their membranes. Identification of these entry sites may make possible blockage of HSV-1 infections through interference with this crucial virus-receptor interaction.

### Gene replacement in adhesion deficiency

**G**ENE replacement therapy may someday be an effective strategy for the treatment of a rare genetic disorder, leukocyte adhesion deficiency (page 1413). Affected individuals suffer from recurrent and life-threatening infections that result from abnormalities in leukocytes; the actual genetic defect—an aberrant CD18 gene—appears to be fairly confined. Wilson *et al.* used genetically engineered retroviruses to insert a normal CD18 gene into cultured cells from a patient with leukocyte adhesion deficiency. Both a structural defect and a functional one were corrected: CD18 molecules appeared on the surface of the patient's cells (from which they had previously been missing) and large aggregates of cells were able to form in an adhesion assay. The next step, which has so far proved technically difficult, will be to transfer this gene into stem cells for human leukocytes so that a new population of competent CD18-bearing cells can be produced in vivo.

■ RUTH LEVY GUYER



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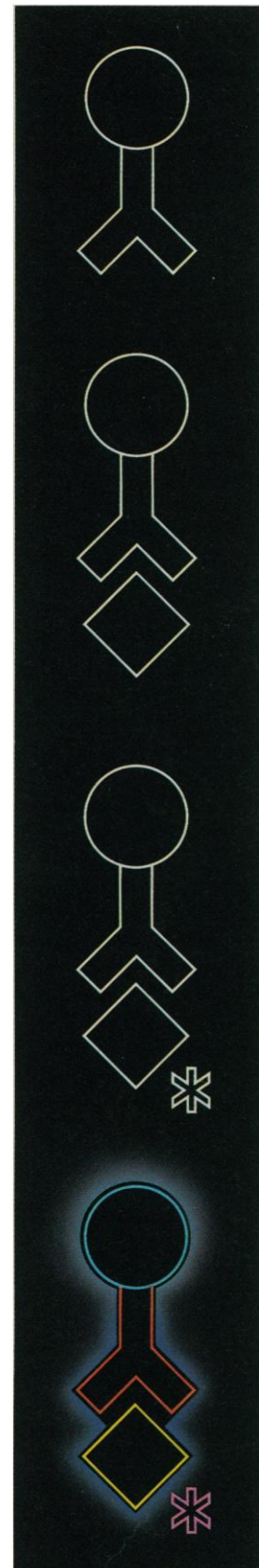
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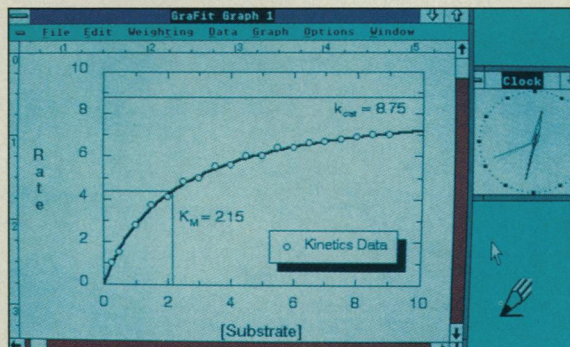
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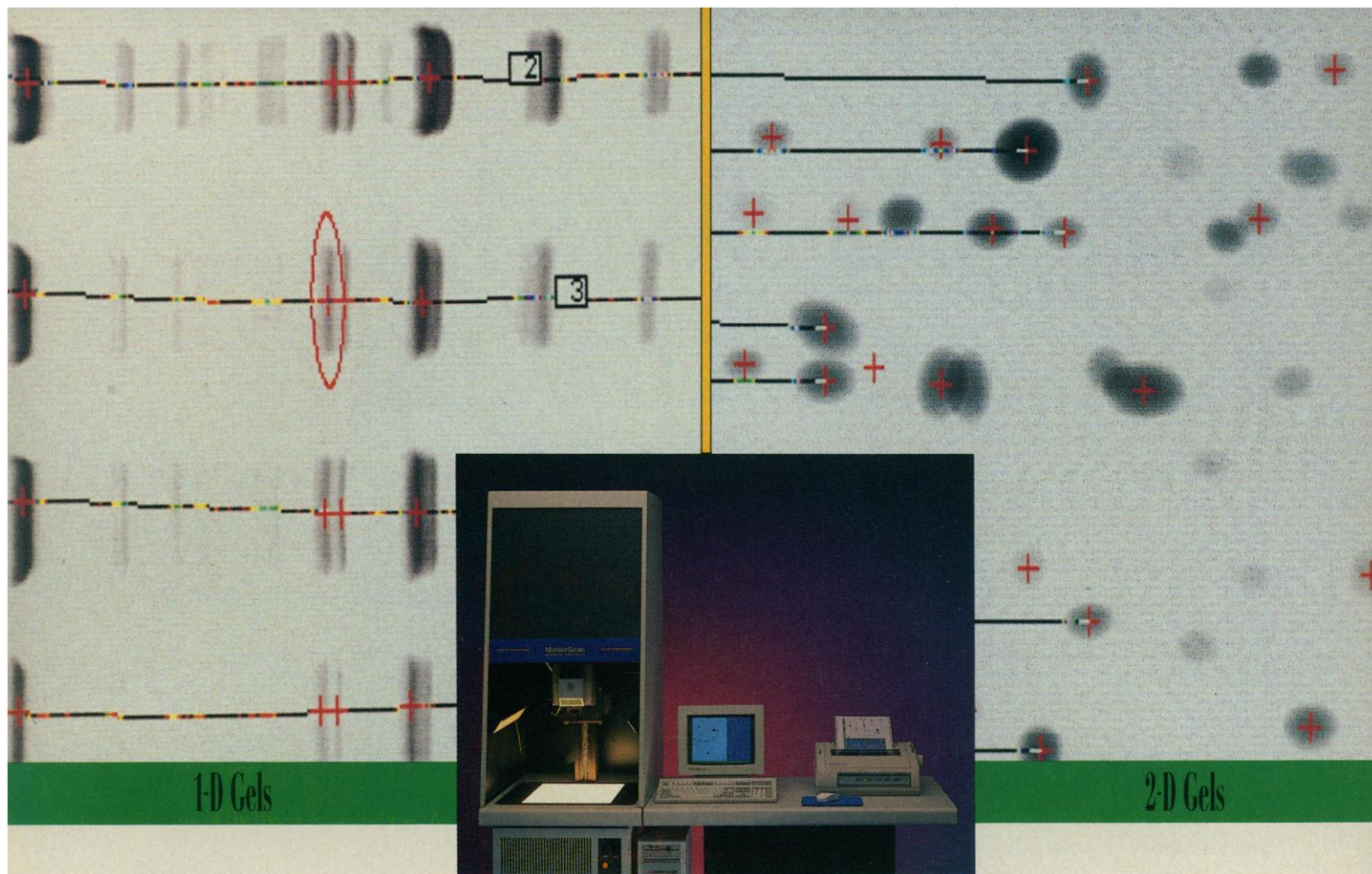


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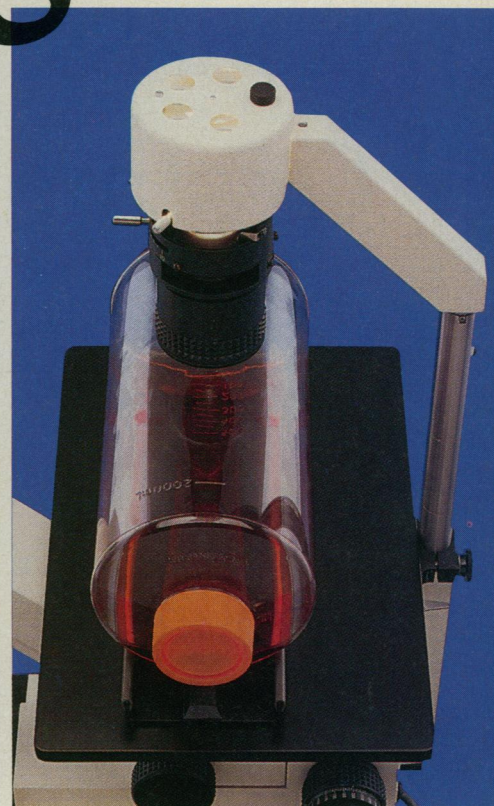
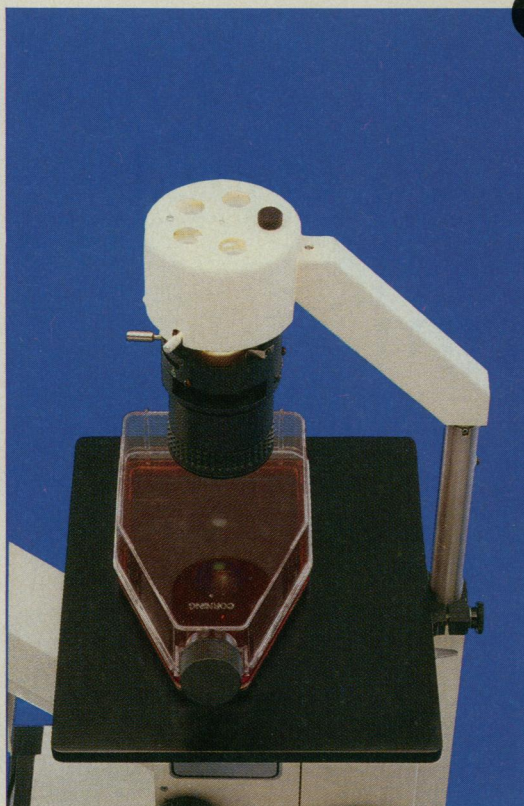
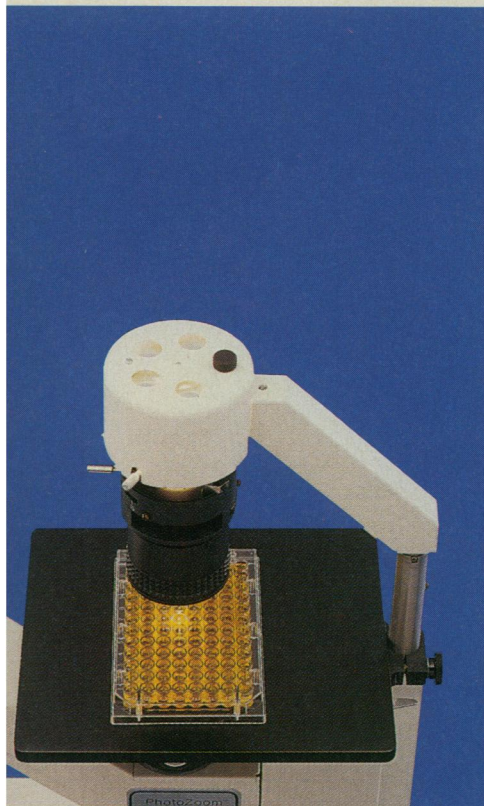
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**Protein Purification**

Sample: Crude extract of *E. coli* lysate (100 µg, 100 µl, 100 µl, 100 µl)  
Column: Cation exchange, sulfonated polystyrene, 10 µm, 250 mm, 390 Å  
Buffer A: 10 mM Tris-HCl, pH 7.5, 20 mM DTT  
Buffer B: 10 mM Tris-HCl, pH 7.5, 20 mM DTT, 1 M NaCl  
Gradient: 0-100% B in 20 min  
Flow rate: 1.0 ml/min  
Cell temp: 4°C  
Detection: 214 nm and 415 nm

**HIV regulatory protein**

The protein, a regulatory protein of the human immunodeficiency virus, contains 84 amino acids, including a Cys-rich cluster and a highly basic region. The fusion protein CII-tat was expressed in *E. coli* and isolated from the soluble cell lysate by cation exchange chromatography at 4°C.

Lysate was loaded through the pump onto the column. The acidic proteins, the majority of the material, were washed through the column followed by gradient elution of the strongly basic CII-tat. The collected fraction was further purified by reverse phase chromatography. Sequencing of the first 20 N-terminal amino acids confirmed the identity of the product.

Chromatogram provided by: Dr. H. V. J. Kelle, Transgene S.A., Strasbourg, France.

**Fast micropurification**

Non-porous resin (NPR) column materials offer the possibility of extremely fast and efficient separation of protein mixtures. The purification of cytochrome c from *R. viridis* required only sonication, centrifugation, and ion exchange chromatography of the crude supernatant.

Previously, the cytochrome had been prepared using low pressure ion exchange chromatography followed by chromatofocusing, requiring more than a full day. The NPR column allowed isolation of 0.5 µg of pure protein in less than 30 minutes.

Chromatogram courtesy of F. Lettspiech, Max Planck Institute, Martinsried, FRG.

**Enzyme purification**

Aspartate aminotransferase (AAT) is a dimeric protein, molecular weight 87,000 and pI 4.6, with pyridoxal phosphate as coenzyme. The enzyme was purified from a strain of *E. coli* containing a multicopy plasmid with the gene for AAT, which results in 100% overexpression of the enzyme.

Cells were sonicated, centrifuged, and insoluble cell debris applied to a strong anion exchange column. Absorption by the coenzyme at 360 nm is used for identifying AAT during elution. This single chromatography purification step recovered active protein 95% pure by the  $A_{280}/A_{360}$  ratio.

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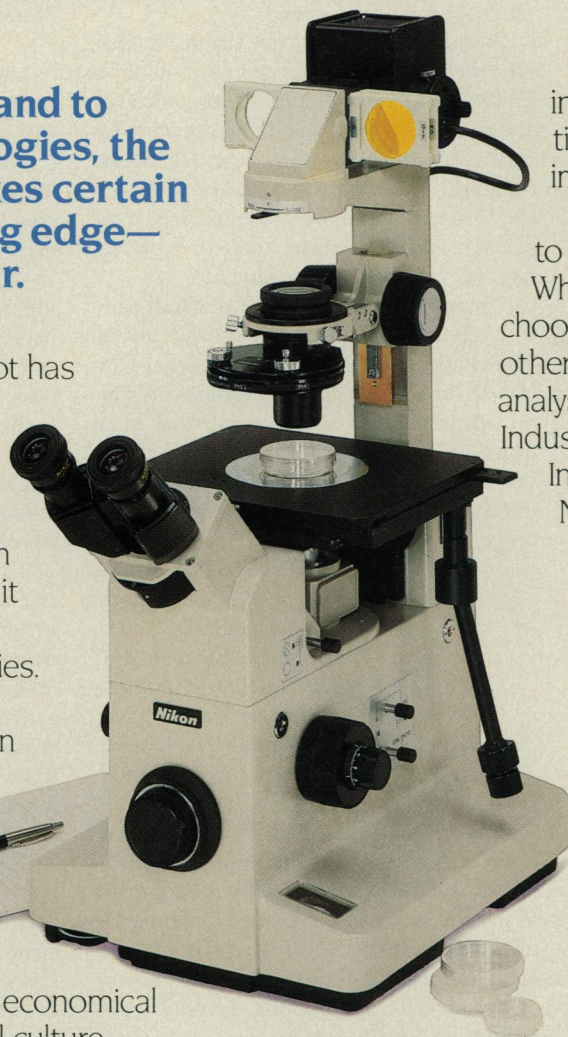
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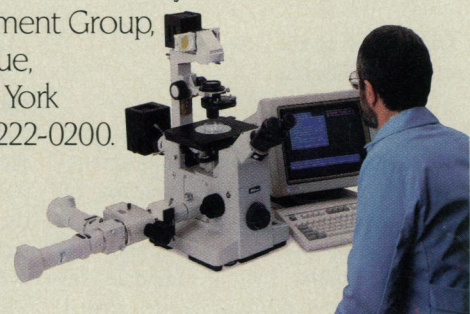
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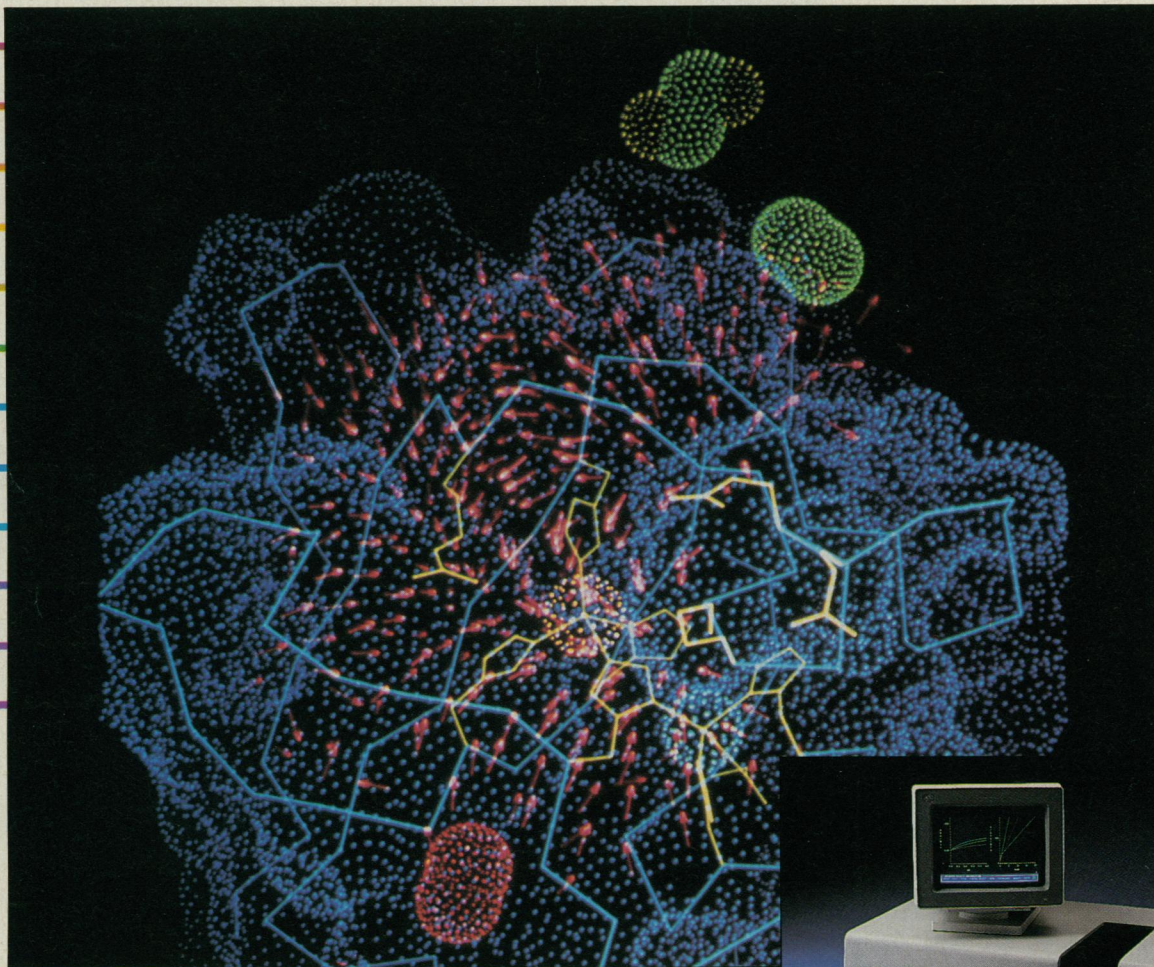


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Superoxide dismutase image by John A. Tainer, Ph.D. Research Institute of Scripps Clinic

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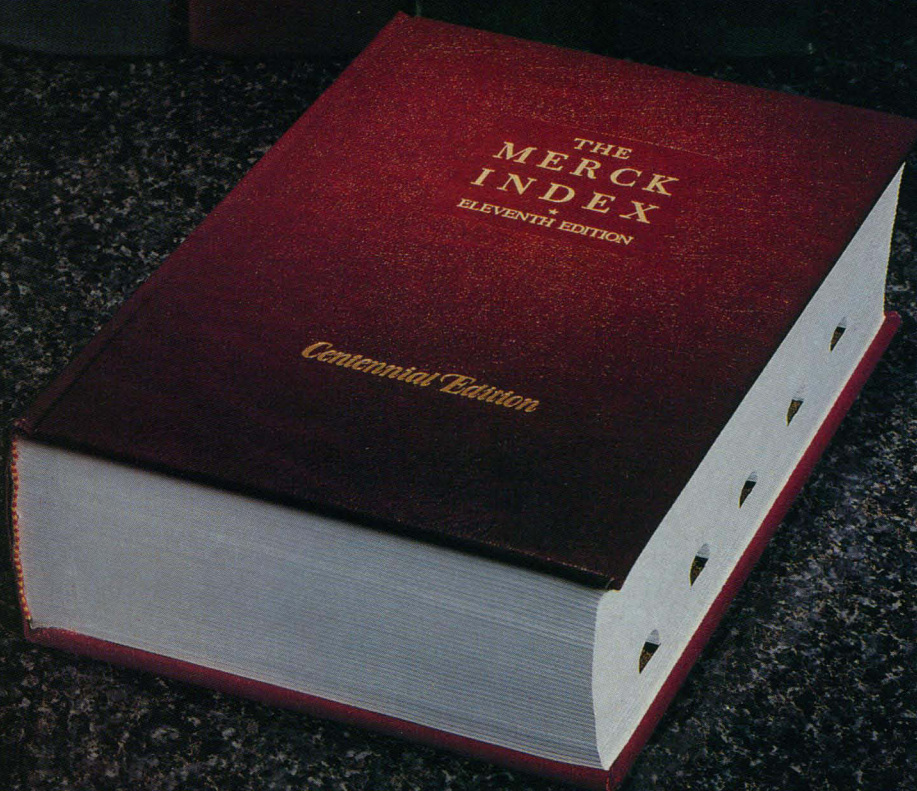
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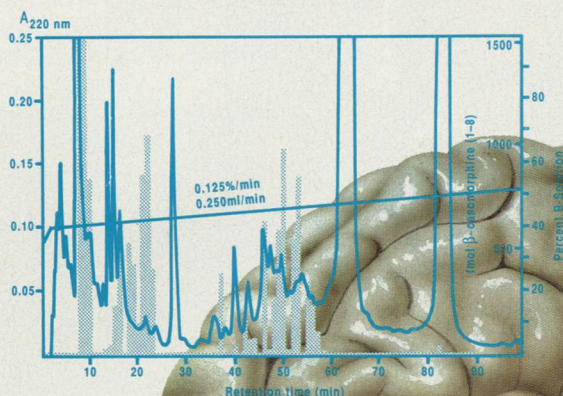
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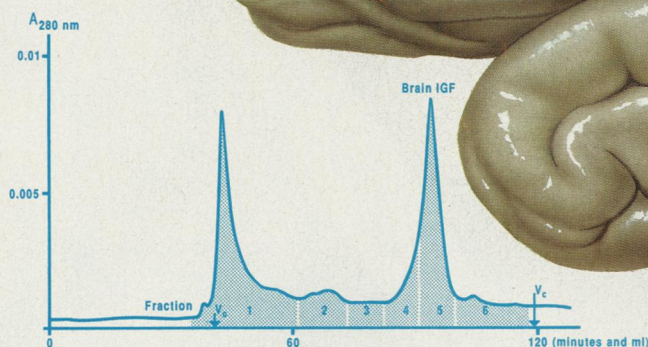
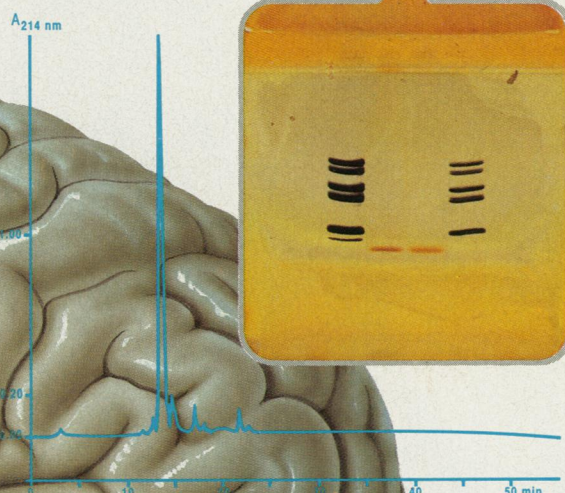
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Purification of  $\beta$ -casomorphine from defatted human milk.  $\beta$ -casomorphines are believed to play a role in postpartum depression. The shaded area shows the  $\beta$ -casomorphine (1-8) immunoreactivity. Column: Analytical Superpac® Pep-S.



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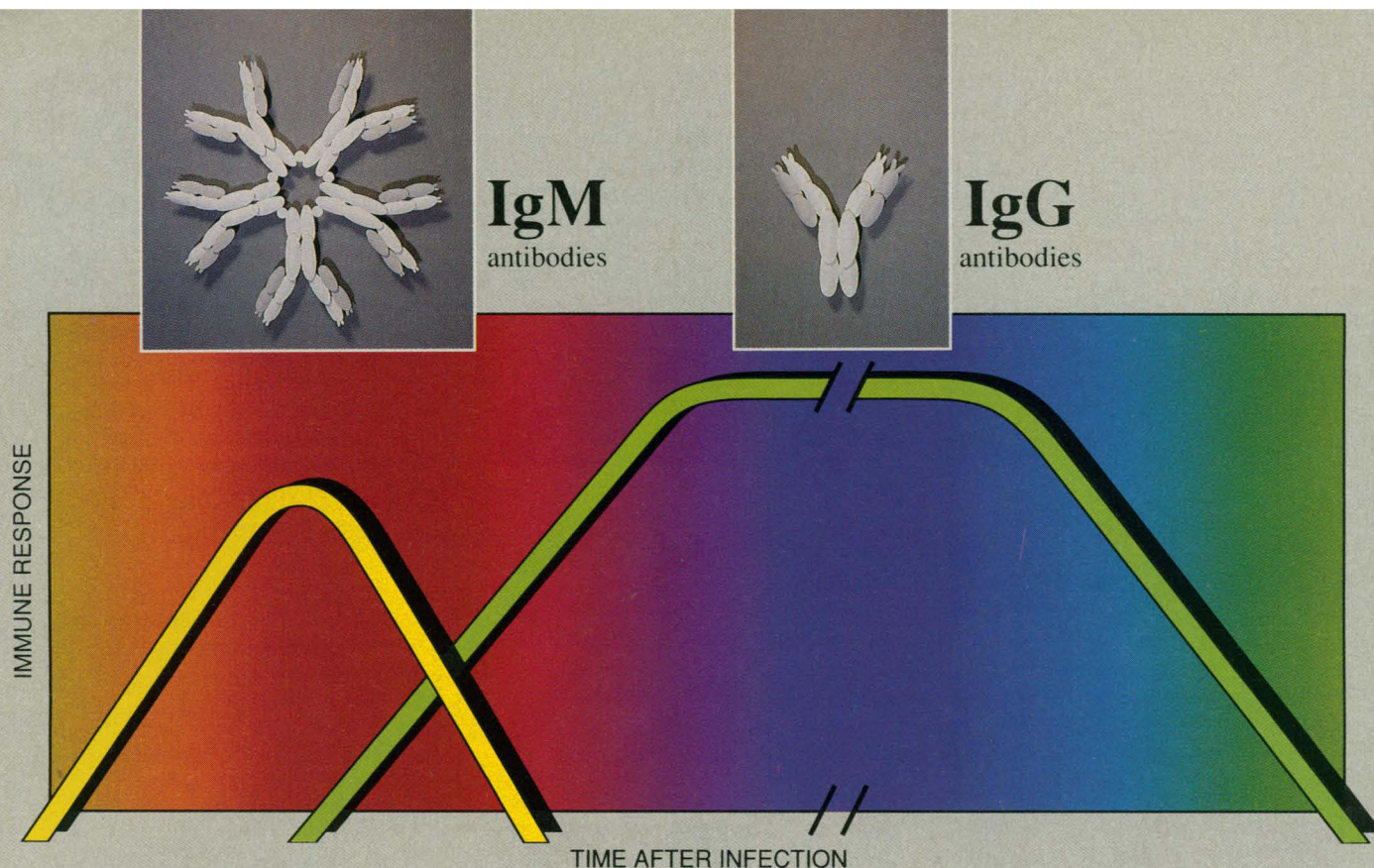
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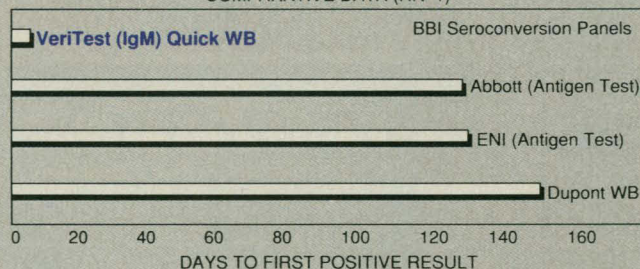
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- Immunochemistry / Immunohistochemistry
- ELISA
- Immunoblotting

### VeriTest Positive Control Reagents

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COMPARATIVE DATA (HIV-1)



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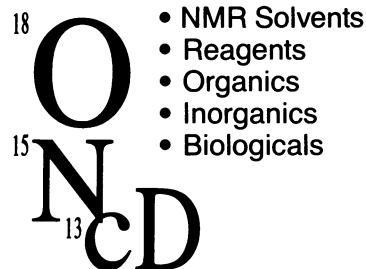
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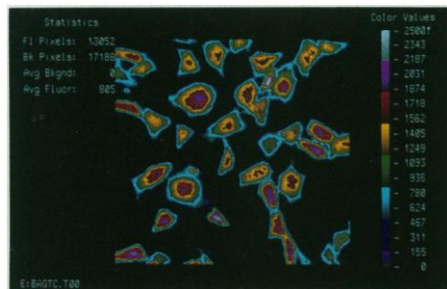
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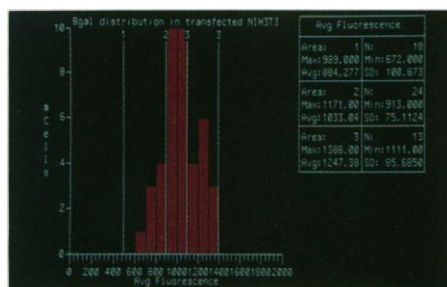
# Reason #7

## "Cutting-Edge" Technology for Clonal Selection

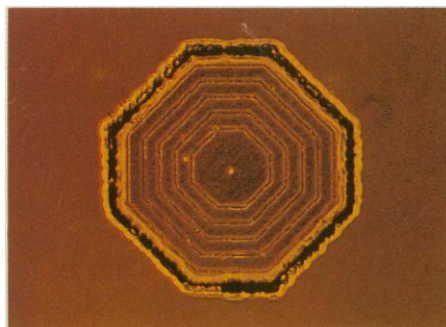
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Two-dimensional, pseudo-color fluorescence image of FDG-labeled, *lacZ*<sup>+</sup>, NIH3T3 cells.



Histogram display of  $\beta$ -galactosidase positive, NIH3T3 cells.



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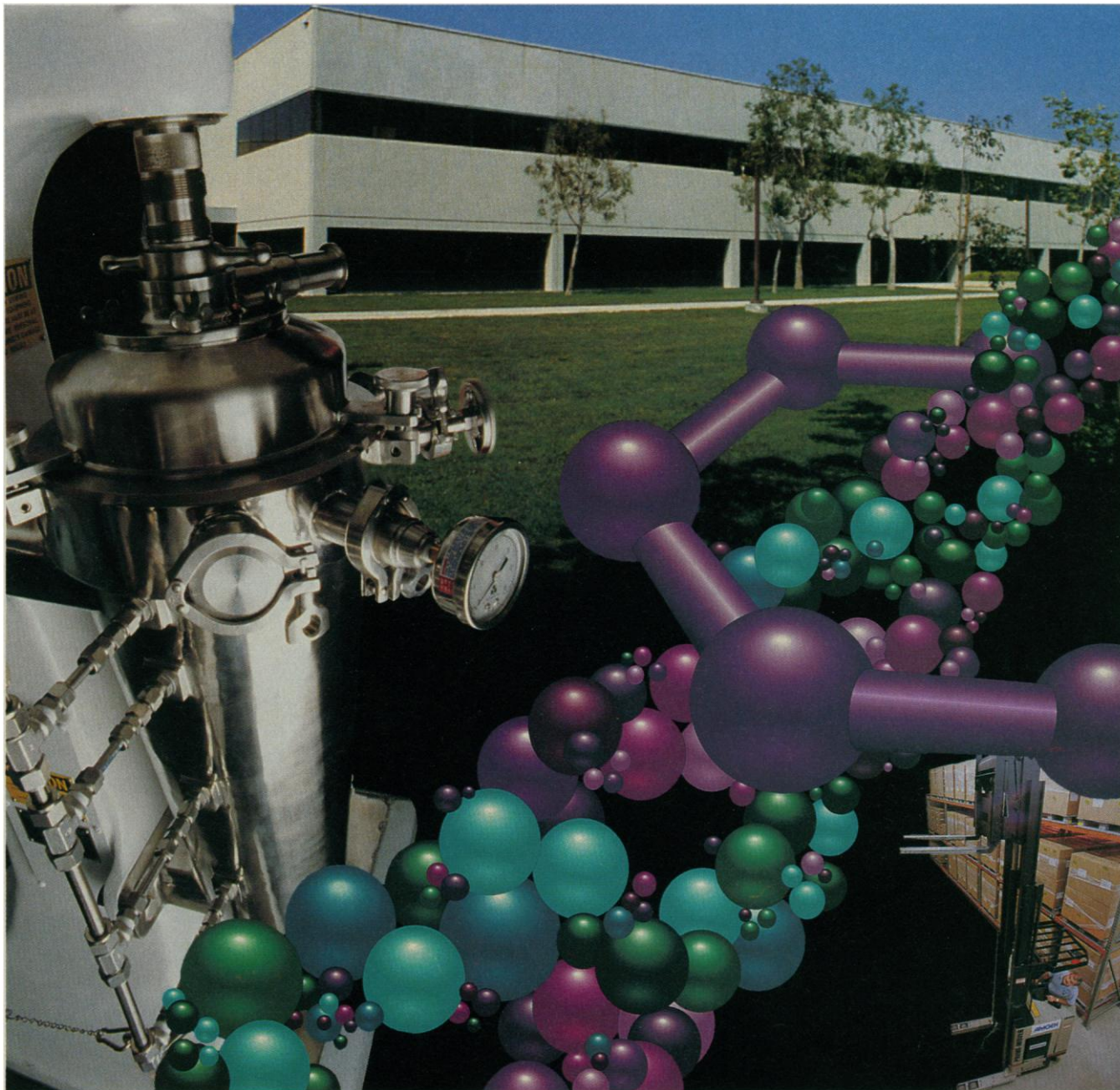
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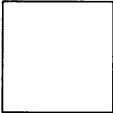
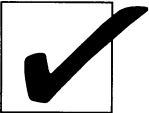



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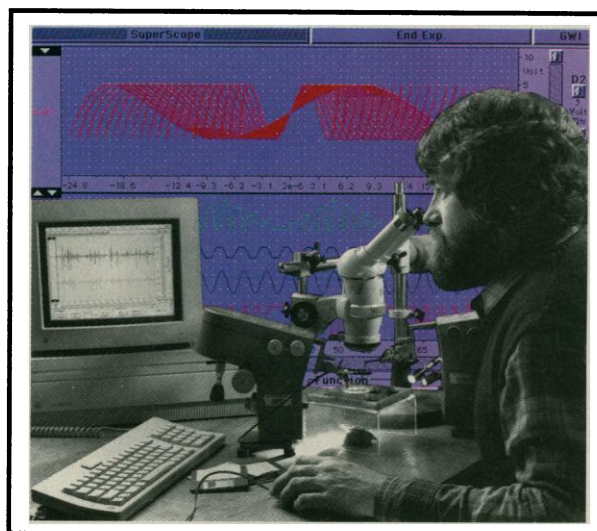
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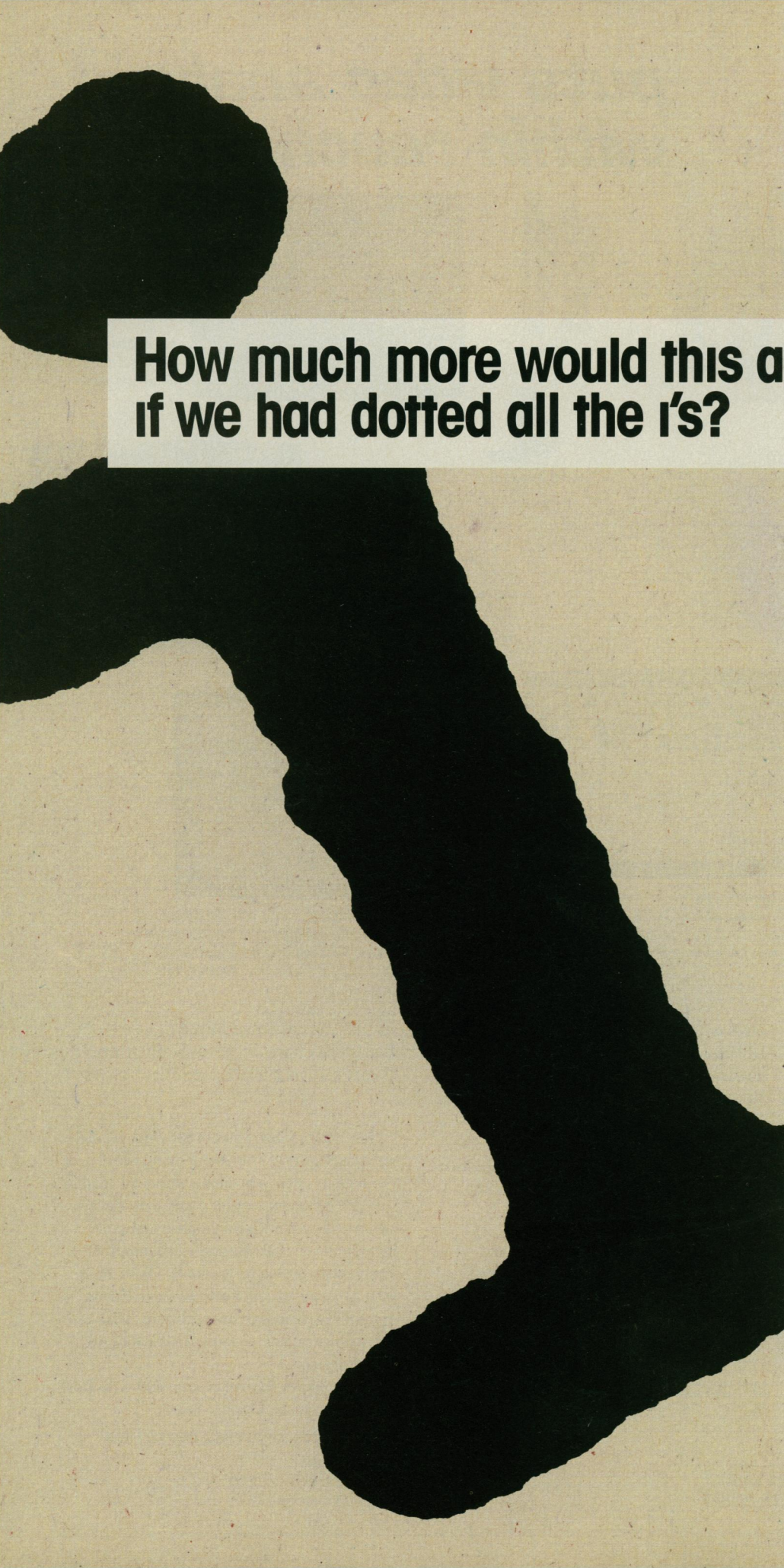


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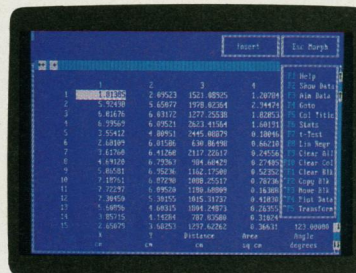
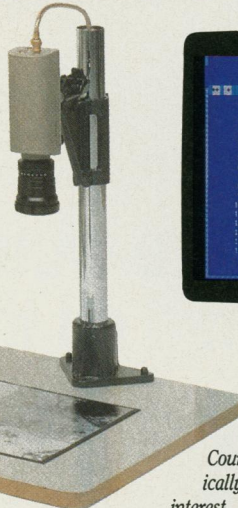
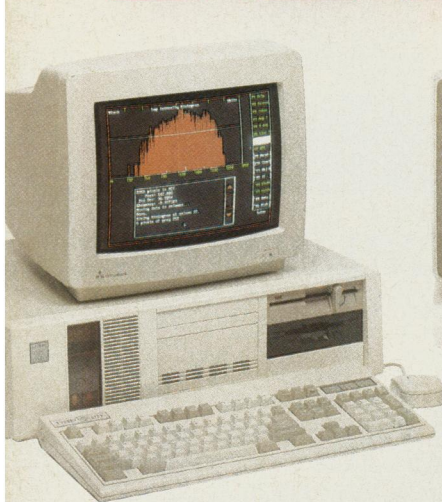
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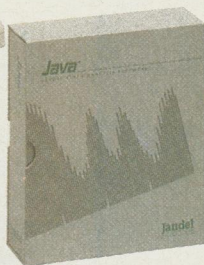
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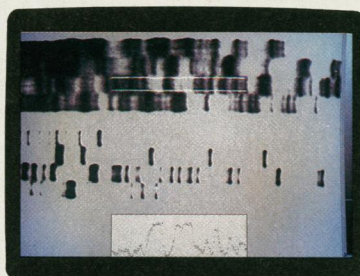
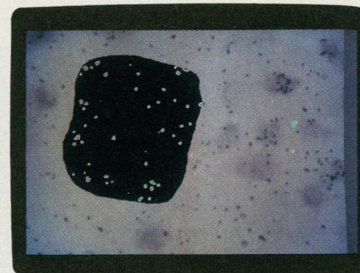
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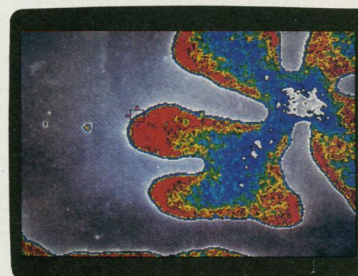
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**Object Counting** — Count objects automatically within any area of interest. JAVA automatically calculates area, perimeter, intensity and x-y location data for each object and places it in the data worksheet.



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#### **Research Grants/Fellowships/Workshops**

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#### **Research Areas of the HFSP**

##### **(A) Basic research for the elucidation of brain functions**

1. *Perception and Cognition*
2. *Movement and Behaviour*
3. *Memory and Learning*
4. *Language and Thinking*

##### **(B) Basic research for the elucidation of biological functions through molecular level approaches**

1. *Expression of Genetic Information*
2. *Morphogenesis*
3. *Molecular Recognition and Responses*
4. *Energy Conversion*

**Research grants:** Major grants for basic research carried out **jointly by research teams in different countries**. The principal applicant must be from one of the eligible countries.\*

**Long-term (3 months-2 years) and short-term fellowships (up to 3 months):** Fellowships for researchers from the eligible countries who wish to do research in foreign countries, or for researchers outside the eligible countries who wish to do research in one of the eligible countries.

**Workshops:** Subsidies for international workshops organized by researchers from the eligible countries.

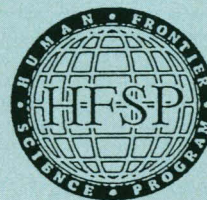
*\*The eligible countries for the current year are Canada, France, F.R.G., Italy, Japan, U.K., and U.S.A. Researchers in non-summit EC member countries may apply through the Commission of the EC on equal terms with the researchers in the eligible countries. (Details in the guidebook)*

**Application Deadline: September 28, 1990**

For research grants, long-term fellowships and workshops, the awards will be announced in April 1991. *Applications for short-term fellowships can be submitted throughout the year.*

Guidebooks and Application Forms may be obtained upon request from the address given below. Please indicate which program activity (Research Grant/Long-term Fellowship/Short-Term Fellowship/Workshop) you are interested in.

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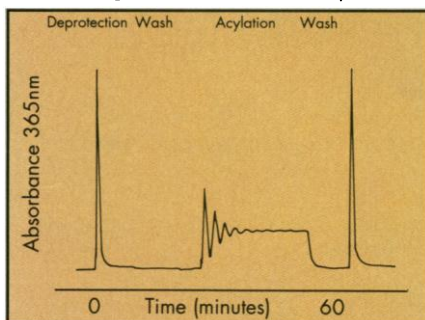
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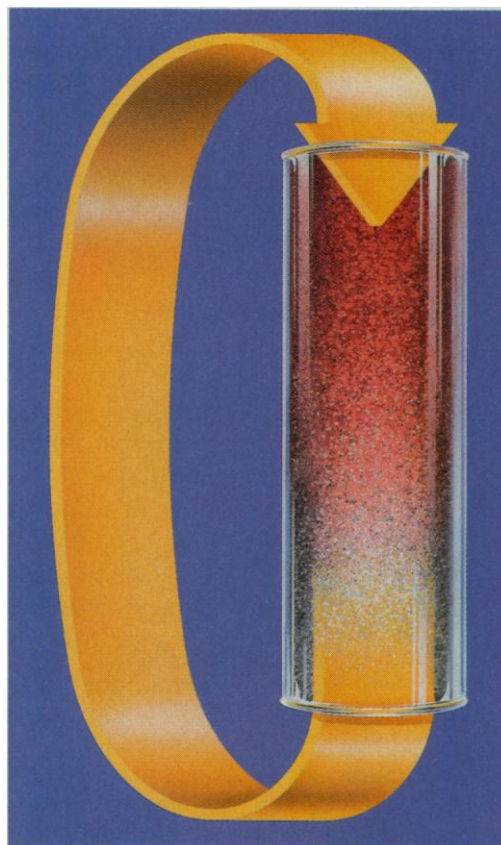
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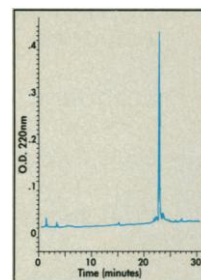
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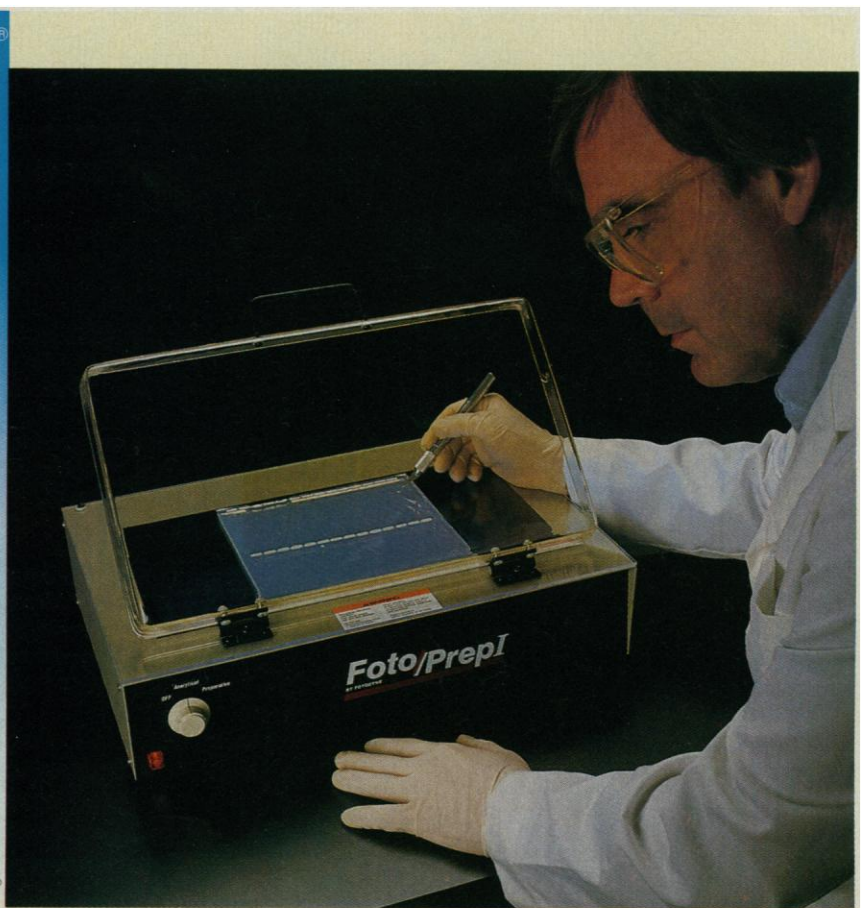
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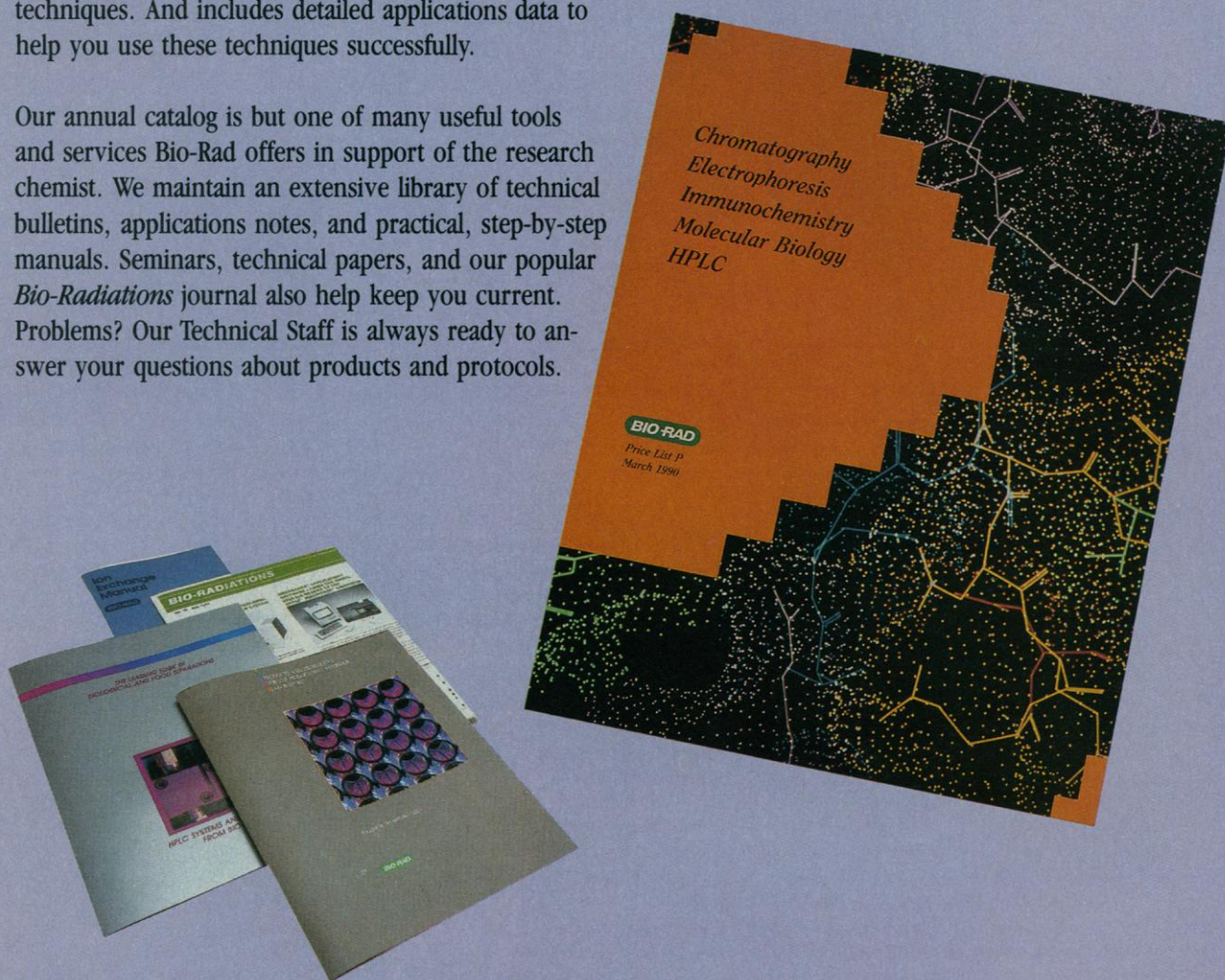
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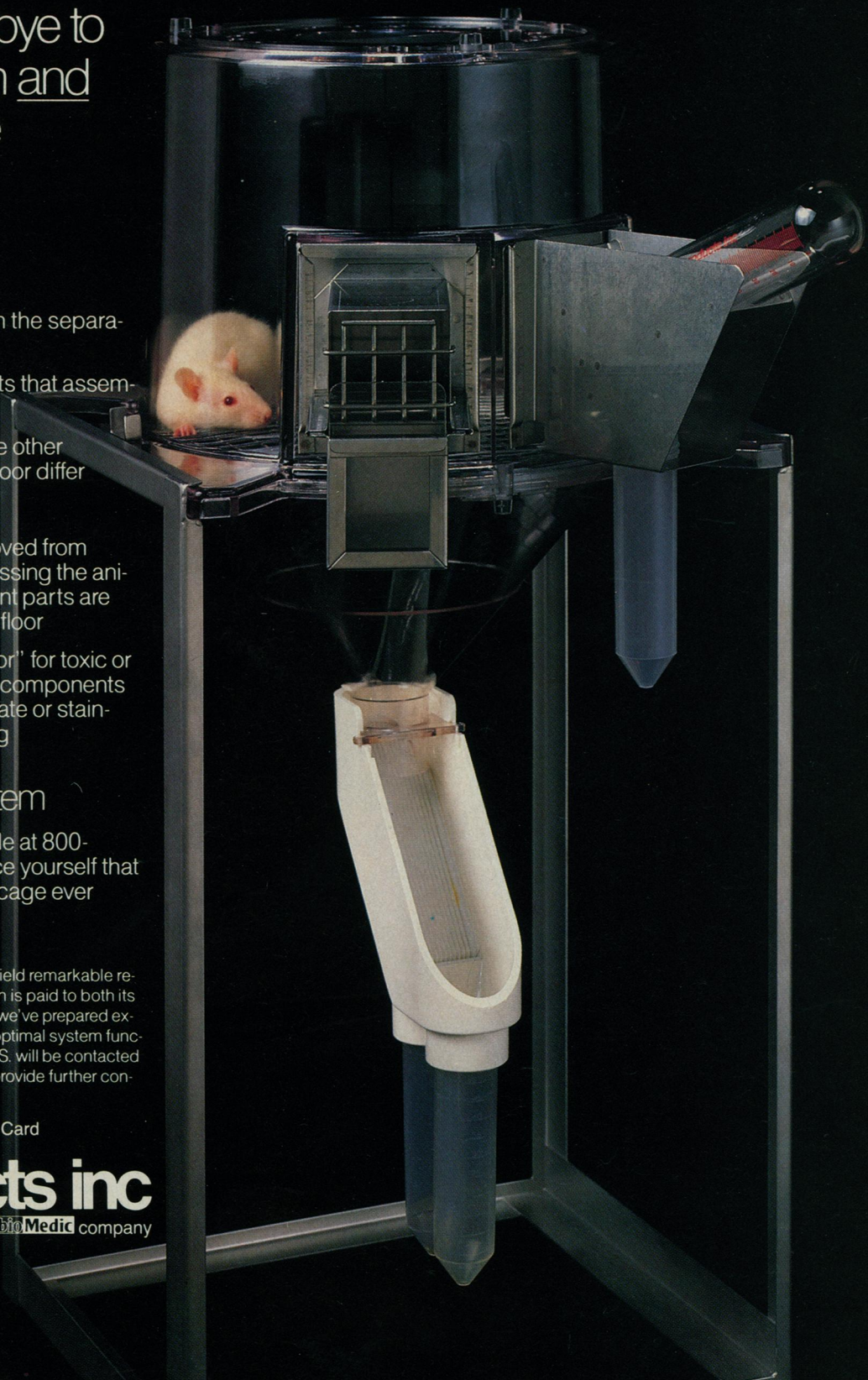
\*This metabolic system does indeed yield remarkable results if, but only if, meticulous attention is paid to both its assembly and operation. Accordingly, we've prepared explicit instructions that will help ensure optimal system functioning. In addition, customers in the U.S. will be contacted by a technical sales representative to provide further consultation as required.

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*Partial List of Speakers*

Walter Bodmer  
David Botstein  
Michael Waterman  
Argiris Efstratiadis  
Frank Ruddle  
Yoshiyuki Sakaki

Mathias Uhlen  
Wilhem Ansorge  
Craig Ventor  
Kay Davies  
Dan Hartl  
Walter Gilbert

Nancy Wexler  
Charles Coutelle  
Charles DeLisi  
Henry Erlich  
David Housman  
Francis Collins

Leroy Hood  
Eric Lander  
Thomas Caskey  
Malcolm Ferguson-Smith  
Richard Myers

*Partial List of Session Topics*

- New techniques in genome research
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- Ethical legal and social issues in the Human Genome project
- Genome projects in non-human organisms
- Handling the flood of Genome information

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**IBEX '90 Conference Chairman: Paul R. Silverman, Ph.D., D. Sci.**, Director of the University of California Systemwide Biotechnology Research and Education Program and Senior Scientist at Lawrence Berkeley Lab

**Session 1: Biotechnology Overview: Status Reports on Genes, Proteins, Carbohydrates and Lipids**  
Session Chair: Howard B. Urovitz, Ph.D., President, Calypse Biomedical Corporation

**Session 2: The Microbiology of Bioremediation: From Laboratory to Field**  
Session Chair: Randall J. von Wedel, Ph.D., President and Director of Research, Cytoculture International

**Session 3: Special Forum: "Inventing to the Need . . . A Purchasing Perspective of Biotech Suppliers"**  
Session Chair: W. R. Clingenpeel, C.P.M., Director of Purchasing Services, Howard Hughes Medical Institute

**Session 4: Infectious Diseases: Achievements in Diagnostics, Advancements in Disease Control**  
Session Chair: Howard B. Urovitz, Ph.D., President, Calypse Biomedical Corporation

**Session 5: Advances in Instrumental Techniques for the Life Sciences**  
Session Chair: Brian Howard, Ph.D., Editor-in-Chief, *International Scientific Communications*

**Session 6: Purification Strategies and Process Design**  
Session Chair: Jan-Christer Janson, Ph.D.  
Scientific Director—Adjunct Professor, Pharmacia LKB

**Session 7: Agriculture and the Seed Industry**  
Session Chair: Robert M. Goodman, Ph.D., Executive Vice President of R&D, Calgene, Inc.

**Session 8: Executive Roundtable—Global Issues Biotech to the Future: Projections**  
Session Co-Chairs: Thomas E. Waller, IBSA President  
Kenneth B. Lee, Jr., National Director, Life Sciences Industry Services, Ernst & Young

### Industry Dinner, Tues., October 23

Our dinner speaker will be Kevin W. O'Connor, a Senior Legal Analyst, who will address the topic  
**"Biotechnology- A View From Capitol Hill"**

Mr. O'Connor is the Project Director for the United States Congress Office of Technology Assessment, which is currently conducting a broad array of studies on aspects of biotechnology that address several concerns of Congress. He will report on these efforts and legislative actions by the 101st Congress.

### Keynote Address, Wed., Oct. 24

***Future Applications of Biotechnology in Agriculture***  
Chancellor Theodore L. Huller, UC Davis

### Special Career Seminar Wed., Oct. 24

***Managing your Biotechnology Career Through the Turbulence of the 1990s***

***To the Year 2000—Biotech Careers On a Changing Course*** Speaker TBA

***What Color is Your Parachute?*** Richard Nelson Bolles, author of the best-selling book of the same name  
***Career Success Through Better Communication*** David Jensen, President, Search Masters International  
***Scientist Versus Manager: How to Decide*** Stan Setwich, Partner, The Emlyn Group  
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### Session 9: Scale-Up of Biochemical Processes: the Growing Challenge

Session Chair: R.B. Roy, Ph.D., Principal Scientist and Director of Application Laboratory, Alpchem Corporation

### Session 10: Biotechnology: Public Perceptions & Public Policies

Session Co-Chairs: Susanne Huttner, Ph.D., Associate Director, UC Education Program, UCLA; Walter Truett Anderson, Ph.D., Environment Editor, *Public News Service*

### Session 11: Analytical Biotechnology—What's New? What's Hot?

Session Chair: William Hancock, Ph.D., Head of Analytical Chemistry & Sr. Scientist, Genentech

### Session 12: Developments in Genomic Mapping & Sequencing

Session Chair: Charles Cantor, Ph.D., Director, Human Genome Center, Lawrence Berkeley Laboratory

### Session 13: Panel Discussion on the Development of Production Scale Biotechnology Operations:

Session Chair: John Sterling, Managing Editor, *Genetic Engineering News*

### Session 14: Regulatory Policy: Drug Development and the Public Interest

Session Chair: Donald Baker, Ph.D., Director Regulatory Affairs, Baxter-Hyland Division

### Session 15: Biocatalytic Production of Chemicals

Session Chair: J. David Rozzell, Ph.D., Director, Industrial Chemicals R&D, Celgene

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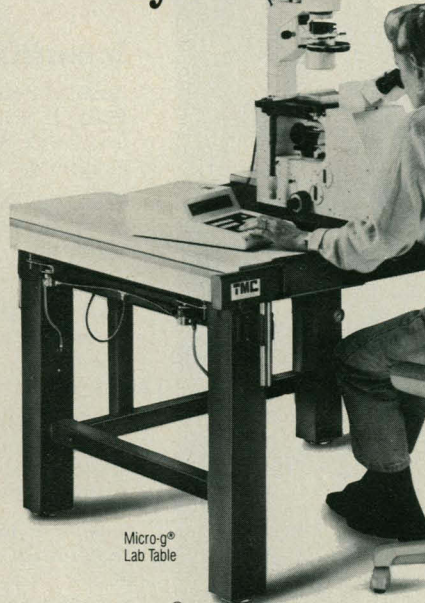


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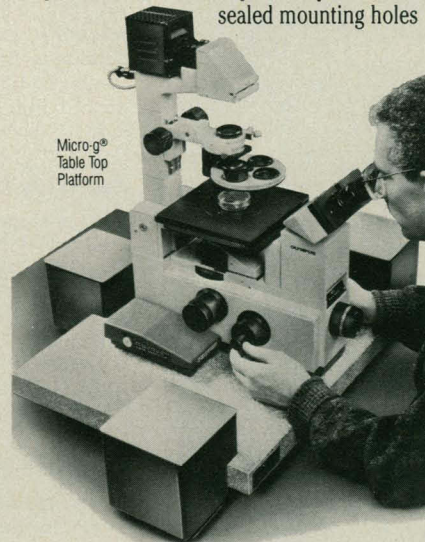
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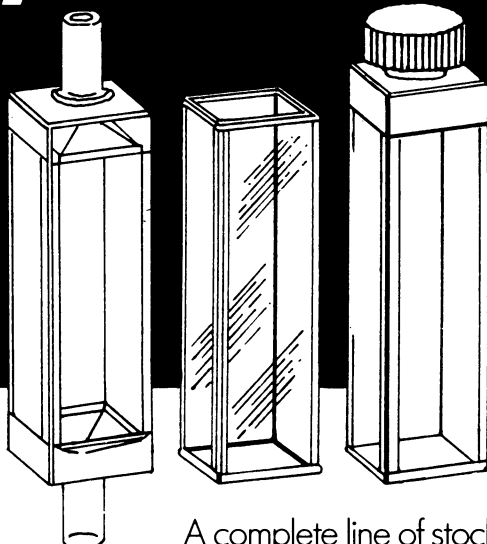


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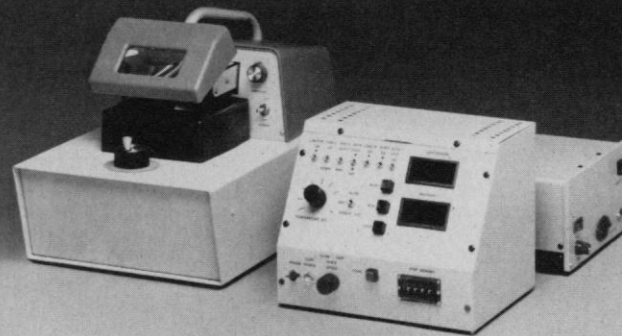
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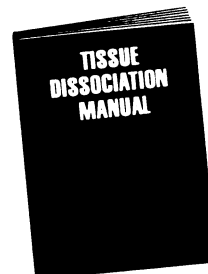


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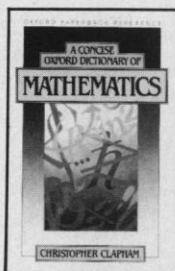
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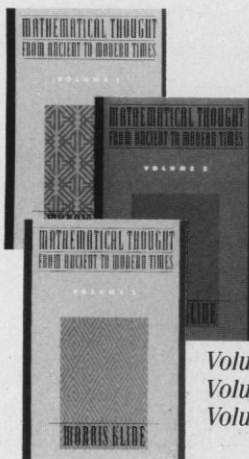


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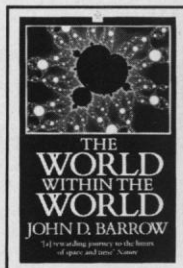
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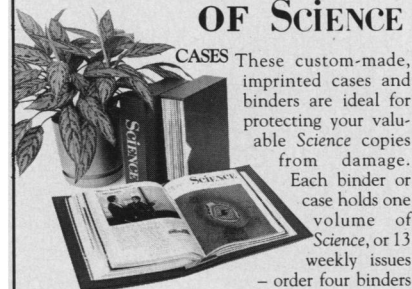
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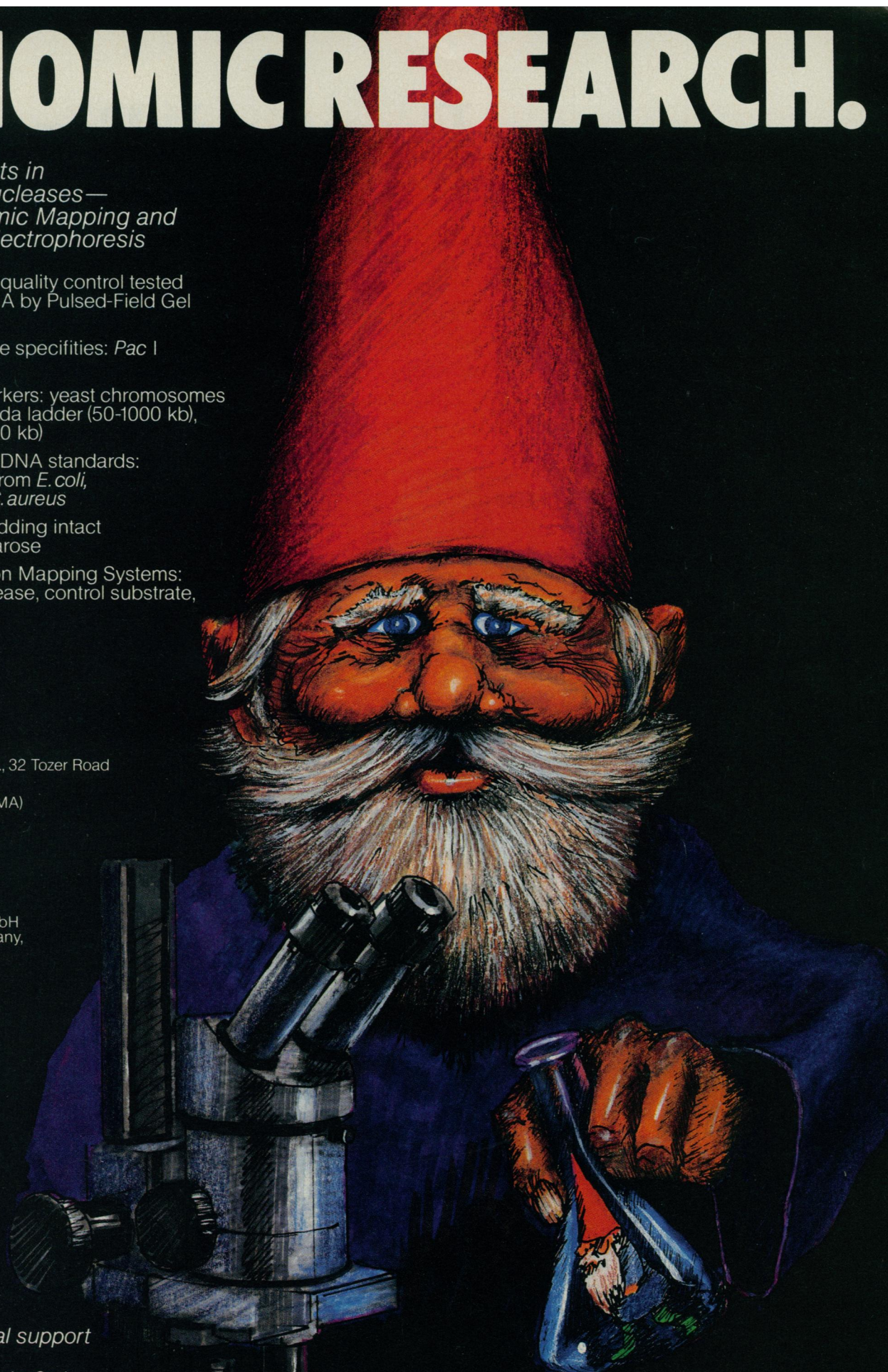
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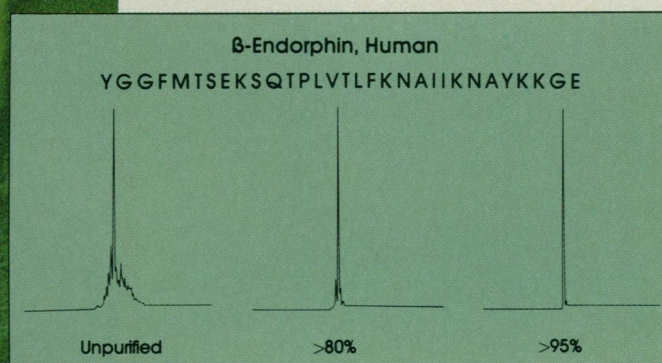
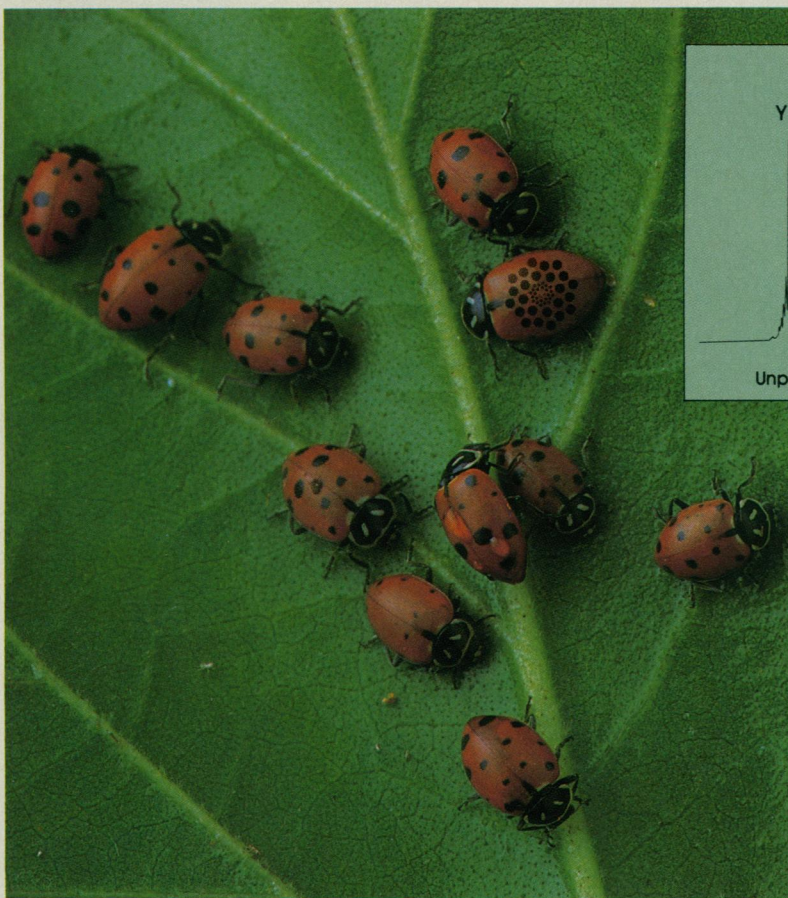




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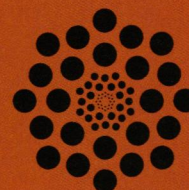
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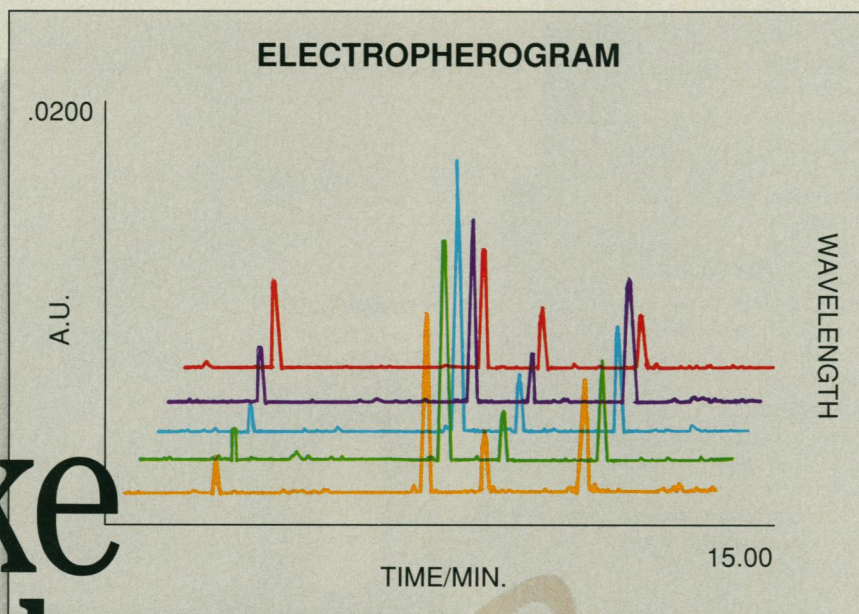
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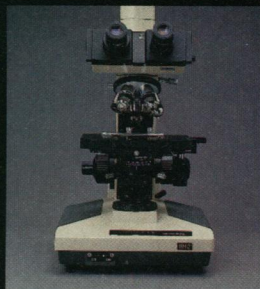
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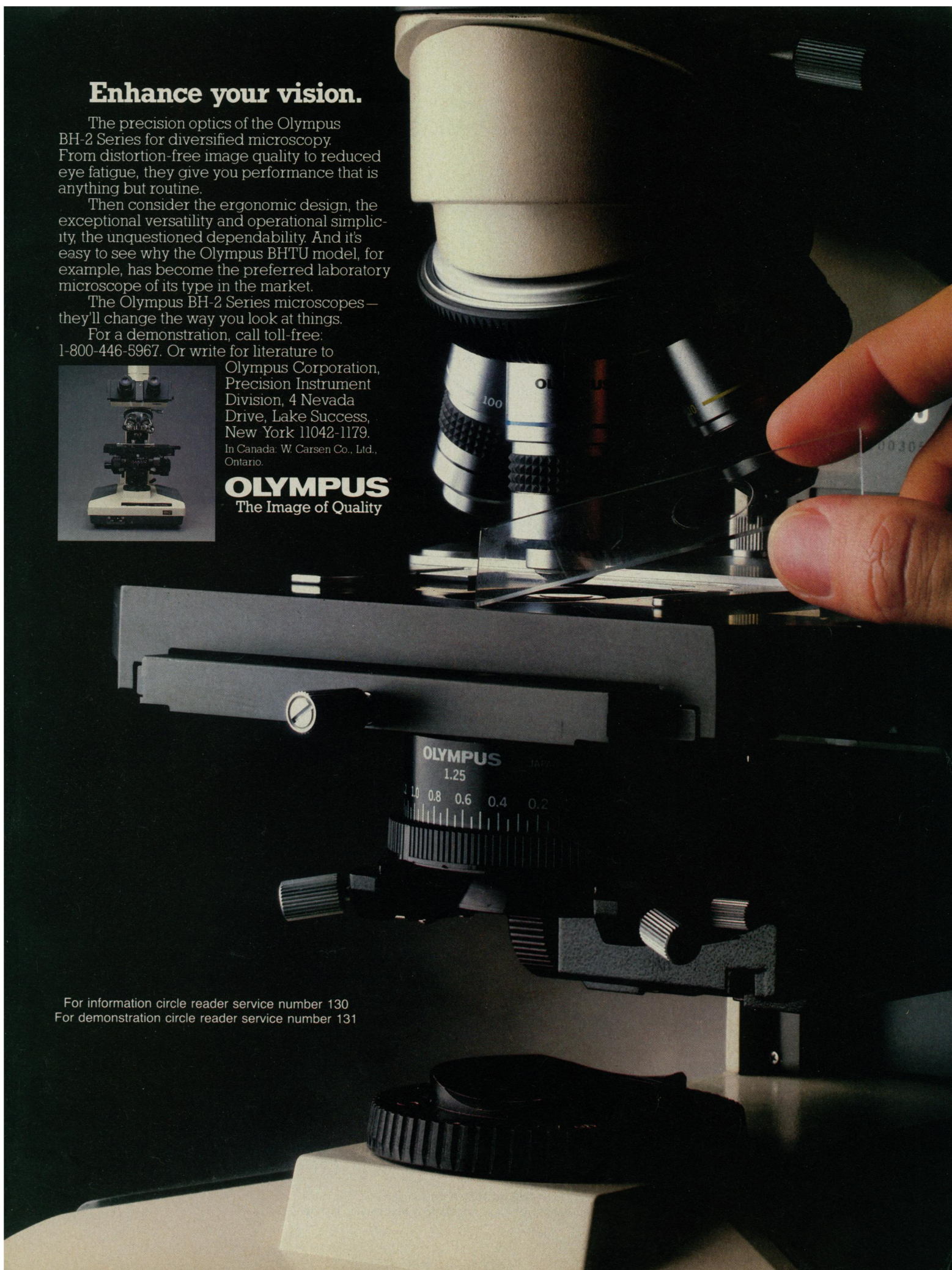
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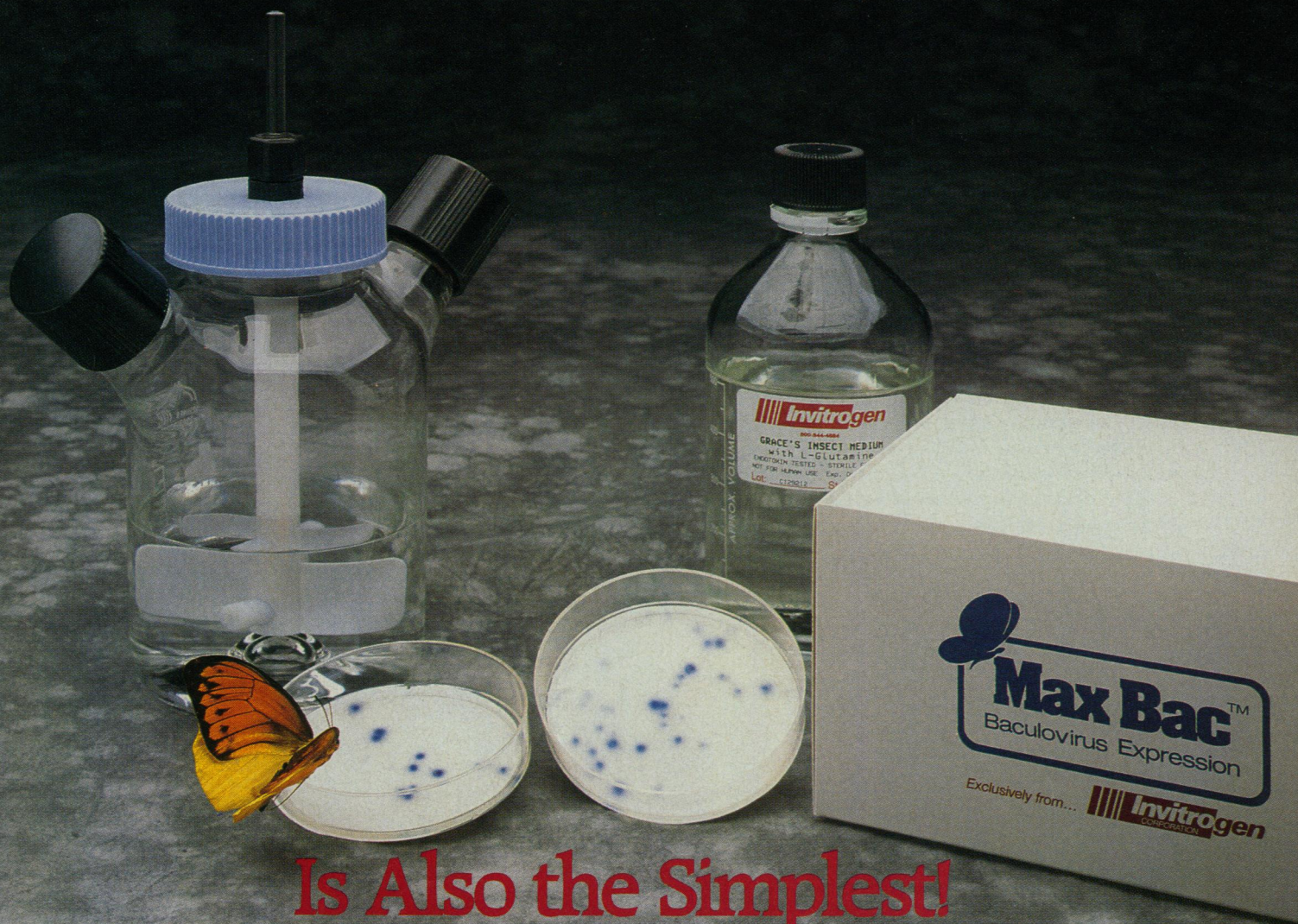


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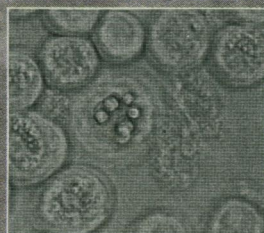


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Luckow, V. and Summers, M. (1988) *Biotechnology* 6, 47-55



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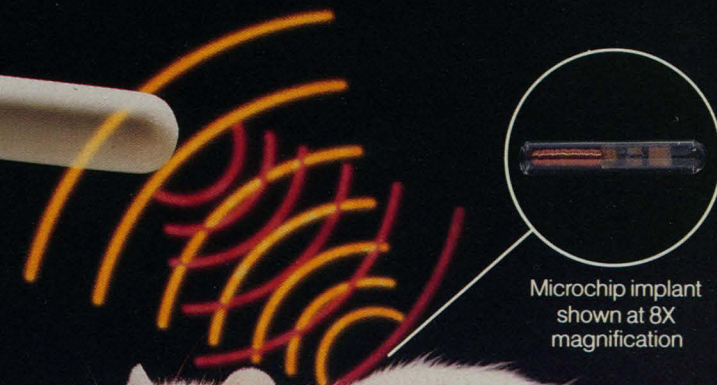
## Background

Some two years ago Bio Medic Data Systems revolutionized laboratory animal identification by introducing an implantable micro-identification device with an encoded number. An interrogation system activates the implantable chip which then transmits its number. In effect: a truly foolproof system akin to adding a unique electronic "universal product code" to each animal.

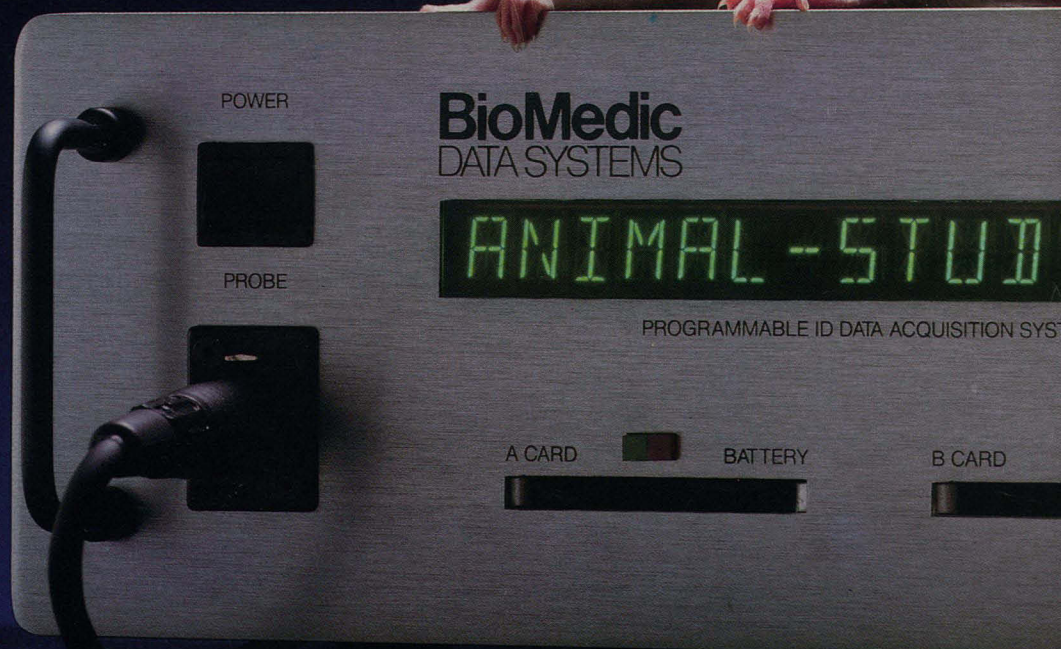
## What are the Benefits?

This simple system obsoletes the traditional ear punching or tagging, toe clipping, and tail tattooing. As such, the age-old labor intensive techniques—in terms of the initial identification, the subsequent reading, and the inevitable re-dos—are replaced by a simple, easy, humane and remarkably efficient system. (A dramatic example: 200 animals can be identified in about 45 minutes.)

In addition: the imprecision of the conventional methods is replaced by *positive animal identification*. Animal misidentification or infection can indeed be catastrophic should they delay, impede, or destroy a crucial investigation. This simple foolproof system now converts ear punching or tagging, toe clipping, and tail tattooing into unacceptable risks... and who needs that when a positive animal identification system is now available!



Microchip implant  
shown at 8X  
magnification



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As a result of a 105-week subchronic evaluation in rats and mice, there have been:

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Microchip is subcutaneously injected into the animal.

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## Who is Using Bio Medic Data Systems' Implantable Micro Identification (IMI)™?

More than 45 organizations now have the Bio Medic Data Systems Implantable Micro Identification including Sandoz Research Institute, Schering Plough, C.I.I.T., General Motors, N.S.I. Technical Services, Stanford University, University of Miami, M.I.T. (Note that the Sandoz Research Institute has submitted the results of the first year of a two-year study for publication.)

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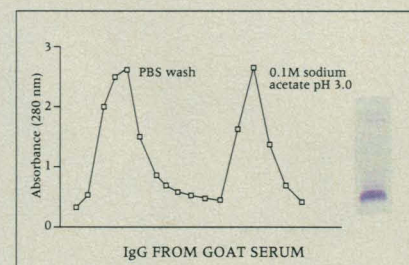
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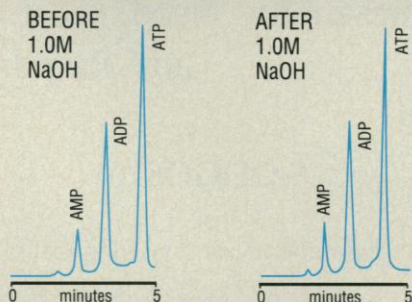
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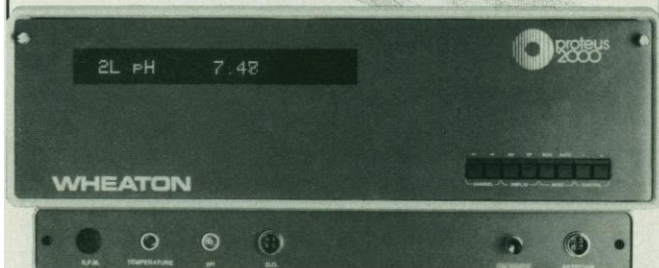
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