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1251 This Week in *Science*

## Editorial

1253 Animal Rights and Animal Wrongs

## Letters

1255 Epidemiologic Investigation: E. H. KASS; S. SHAPIRO; A. R. FEINSTEIN

## News & Comment

- 1279 Europe Recognizes the Ozone Threat
- 1280 Pesticides and Kids
- 1281 NASA's \$60,000 Epoxy Drops
- 1282 Chinese Bar Physicist from Bush Dinner  
NAS Weighs in With 7-Lb Diet Guide
- 1283 GM, Hughes Settle Stock Fight  
An Arbitrator for Space Policy
- 1284 France Introduces Bioethics Law  
Germany Enters Hypersonic Race
- 1285 Japan Faces Big Task in Improving Basic Science
- 1287 The Drug Czar: No "Walter Wallflower"

## Research News

- 1288 Image Capture by Computer
- 1289 How Females Entrap Males
- 1290 Is Something Strange About the Weather? ■ Where Strange Attractors Lurk
- 1293 Seeing Cracks in Three Dimensions
- 1294 Hidden Complexities in the Risks of Extinction

## Articles

- 1313 Is the Stock Market Efficient? B. G. MALKIEL
- 1319 Model of the Origin of Rhythmic Population Oscillations in the Hippocampal Slice: R. D. TRAUB, R. MILES, R. K. S. WONG

## Research Articles

- 1325 Hydrogen Tunneling in Enzyme Reactions: Y. CHA, C. J. MURRAY,  
J. P. KLINMAN
- 1330 Receptor and Antibody Epitopes in Human Growth Hormone Identified by  
Homolog-Scanning Mutagenesis: B. C. CUNNINGHAM, P. JHURANI, P. NG,  
J. A. WELLS

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**COVER** Photograph of a dwarf plant of *Arabidopsis thaliana* isolated from a population of transformants that had been generated by infecting seeds with *Agrobacterium*. Genetic analysis indicates that the recessive mutation that caused this phenotype is due to the insertion of a T-DNA from *Agrobacterium* into a gene that in the homozygous state confers dwarfism to the plant. The plant pictured was 3.5 centimeters in height. The portion pictured is 3 centimeters in height. See page 1351. [Photograph by DeVere Patton, senior photographer, E. I. du Pont de Nemours & Company, Wilmington, DE 19898]

## Reports

- 1337 Plate Tectonics 2.5 Billion Years Ago: Evidence at Kolar, South India: E. J. KROGSTAD, S. BALAKRISHNAN, D. K. MUKHOPADHYAY, V. RAJAMANI, G. N. HANSON
- 1340 The Axial Oxygen Atom and Superconductivity in  $\text{YBa}_2\text{Cu}_3\text{O}_7$ : S. D. CONRADSON AND I. D. RAISTRICK
- 1343 Honeyguides and Honey Gatherers: Interspecific Communication in a Symbiotic Relationship: H. A. ISACK AND H.-U. REYER
- 1346 Structure of Recombinant Human Renin, a Target for Cardiovascular-Active Drugs, at 2.5 Å Resolution: A. R. SIELECKI, K. HAYAKAWA, M. FUJINAGA, M. E. P. MURPHY, M. FRASER, A. K. MUIR, C. T. CARILLI *et al.*
- 1351 A Dwarf Mutant of *Arabidopsis* Generated by T-DNA Insertion Mutagenesis: K. A. FELDMAN, M. D. MARKS, M. L. CHRISTIANSON, R. S. QUATRANO
- 1354 Effect of Antisense *c-raf-1* on Tumorigenicity and Radiation Sensitivity of a Human Squamous Carcinoma: U. KASID, A. PFEIFER, T. BRENNAN, M. BECKETT, R. R. WEICHSELBAUM, A. DRITSCHILLO, G. E. MARK
- 1357 Selection for Precise Chromosomal Targeting of a Dominant Marker by Homologous Recombination: J. R. DORIN, J. D. INGLIS, D. J. PORTEOUS
- 1360 Phylogenetic Stains: Ribosomal RNA-Based Probes for the Identification of Single Cells: E. F. DELONG, G. S. WICKHAM, N. R. PACE
- 1363 Nucleotides in Yeast tRNA<sup>PHE</sup> Required for the Specific Recognition by Its Cognate Synthetase: J. R. SAMPSON, A. B. DiRENZO, L. S. BEHLEN, O. C. UHLENBECK
- 1367 Free Calcium at Rest During "Catch" in Single Smooth Muscle Cells: N. ISHII, A. W. M. SIMPSON, C. C. ASHLEY

## Book Reviews

- 1373 Topobiology, reviewed by J. GERHART ■ Biology and the Mechanics of the Wave-Swept Environment, M. R. PATTERSON ■ Remembering Reconsidered, D. L. SCHACTER ■ Some Other Books of Interest ■ Books Received

## Products & Materials

- 1381 Superconductor Powder ■ Miniature Electrophoresis System ■ Spectrofluorometer ■ Radial Chromatography Columns ■ Graphics Software ■ Energy-Filtering Electron Microscope ■ Motion Analysis System ■ Recombinant Protein A-Protein G Fusion ■ Literature

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## This Week in SCIENCE

### Hydrogen tunneling

**Q**UANTUM-MECHANICAL effects are not generally associated with enzymology; however, Cha *et al.* theorize and demonstrate that the phenomenon of nuclear tunneling can make a significant contribution to the mechanism of an enzyme reaction under normal conditions (page 1325). Tunneling occurs when a particle passes through an energy barrier that it could not overcome classically. Although electron tunneling is known to occur with redox proteins, larger particles such as nuclei have not been observed to tunnel in biologic systems at room temperature. Yet, during catalytic conversion of benzyl alcohol to benzaldehyde by the yeast enzyme alcohol dehydrogenase—a reaction for which the rate-limiting step is hydrogen transfer—nuclear tunneling did occur; this was demonstrated experimentally through a comparison of the kinetics of transfer for protium, deuterium, and tritium, three hydrogen isotopes that differ in atomic mass. The observation of tunneling can provide information about energy barriers that affect catalytic reactions and insights into how enzymes facilitate catalysis.

### Homolog-scanning mutagenesis

**A** physical map of the distribution of protein epitopes—determinants that bind receptors or antibodies—has been generated by homolog-scanning mutagenesis (page 1330). In the prototype experiment, Cunningham *et al.* evaluated the locations of epitopes on a synthetic human growth hormone (hGH) that has receptor and antigenic properties identical to those of the natural protein. The hGH gene was “mutagenized” by insertions of small segments from genes which encode proteins that are structurally analogous to hGH but that bind poorly to the hGH receptor or to monoclonal antibodies that recognize hGH epitopes. Despite the substitutions, the chimeric proteins were able to fold properly; and, although substitutions occurred throughout the chain, discrete

patches on the folded protein accounted for lost binding properties. The epitopes are assembled from discontinuous sequences brought together in the globular molecule when it folds. This technique is likely to have wide application for linking structure and function in proteins for which sequence information is known or for which homologous but functionally different proteins are available.

### Ancient plate tectonics

**E**VIDENCE from the Dharwar Craton (southern India) presented by Krogstad *et al.* suggests that plate tectonic processes, which have been responsible for building continents for at least the last 600 million years, were already shaping the earth's crust in the Archean, about 2.5 billion years ago (page 1337). Age and isotopic differences between rocks on the eastern and western sides of the craton suggest that the rocks in each region were derived at different times (the west is older) and from different mantle sources. Marking the juncture of the two terranes is the Kolar Schist Belt, a suture zone that runs north and south for at least 80 kilometers. The style of crustal growth is indicative of plate tectonic activity, and the two terranes apparently collided about 2.42 billion to 2.53 billion years ago. An understanding of the forces that affected the early evolution of the earth's crust provides a basis for interpreting the early histories of the other terrestrial planets and of the moon.

### Honeyguides and honey gatherers

**I**N their searches for honey, nomadic African peoples communicate with birds, and this communication benefits both the gatherers and their guides (page 1343). Boran honey gatherers are led by honeyguides (birds of the group *Indicator indicator*) to colonies of bees. With the assistance of the honeyguides, the gatherers' average search times for new nests are reduced from

8.9 to 3.2 hours. The birds benefit too, because the Borans open the (otherwise inaccessible) nests, making the larvae and wax available to the guides; furthermore, fires made by the Borans provide a protective smoke screen for the birds against stinging bees. The complex interspecies communications, described by Isack and Reyer, include the Borans' special whistle to attract honeyguides and their subsequent shouts and knocks (on dry wood) to sustain communications; the honeyguides call and make restless movements to attract the gatherers and, throughout their searches, make sounds, fly, and perch in ways that the Borans understand as providing updated information about the location of the nest. This interdependence of bird and man may be a dying phenomenon as beekeepers replace bee gatherers and as other foods are substituted for honey in the diets of African peoples.

### Phylogenetic stains

**F**LUORESCENT oligonucleotide probes are proving to be reliable markers for identifying and distinguishing among microorganisms (page 1360). The synthetic oligonucleotides—each having 17 to 34 bases arranged in a sequence that is complementary to the sequence of a known ribosomal RNA species—are applied to microbes fixed on slides; the probe hybridizes with the microbes' ribosomal RNA. With fluorescence microscopy, fluorescent tags can be observed and distinguished (when more than one is used). The types of probes that can be developed range from universal probes (those that have sequences complementary to ribosomal RNA shared by all microbes), probes that distinguish among the primary kingdoms (eukaryotes, eubacteria, and archaebacteria), and probes specific for smaller subgroups or for individuals. Examples of how such probes resolve microorganisms are illustrated in the studies of DeLong *et al.* Because the ribosomal RNA content of a microorganism is proportional to its growth rate, this technique can, in principle, provide data on how fast cells are growing.

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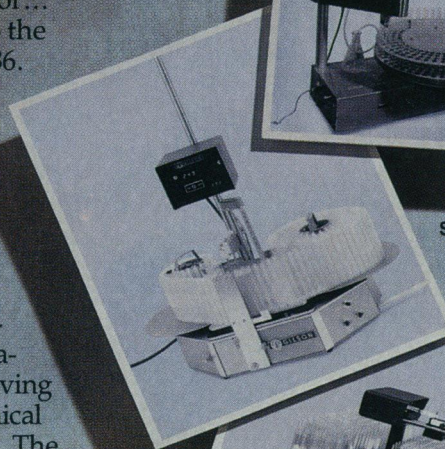
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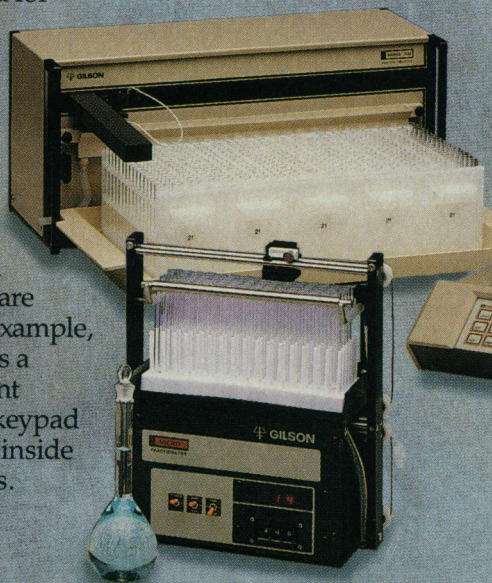
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## Animal Rights and Animal Wrongs

At a recent demonstration against a new animal care building at the University of California, animal rightists put up a sign that said, "These animals are to be used by torturers, prisoners, and murderers." Torturers? Some procedures that these animals undergo, such as removal of blood samples and surgery under anesthesia, are no different from procedures performed on humans at any hospital. Prisoners? The cages in a research laboratory are no different from the cages in a humane society or a veterinary hospital. Murderers? A strange term to apply to animal experimenters when a conservative estimate is that pounds and shelters kill a hundred stray cats and dogs for every such animal sacrificed in research. Such perversion of language and misleading rhetoric are among the reasons that many view with increasing skepticism the argument that animal rights organizations are "sincere," even though many of their followers certainly are. The time has come to take important steps to protect both research and the image of humane societies as legitimate protectors of kindness to animals.

Ten years ago, before the current wave of legislation, it could fairly be said that some animal experiments were done improperly, in inadequate facilities, or with inappropriate supervision. These days are largely past. The current protocols for care and treatment of animals are so stringent that most modern animal facilities are models for responsible and considerate treatment. Yet the ever-escalating demands to set standards beyond the norm even for hospitals are leading scientific groups to the conclusion that the agenda is not the prevention of cruelty in research, but rather the absence of all animal research.

The first step in clarifying the roles of the participants must come from the animal rights organizations. They must state clearly whether they accept the necessity of using animals in any kind of research or not at all. If they choose "research yes, cruelty no," they must be willing to take a stand against extremists who denounce the very facilities that research institutions have developed at great expense to ensure humane handling of animals. If animal rights organizations choose the "no animal research" alternative they should then be willing to face the AIDS patient, the cystic fibrosis patient, the cancer patient, and present their case that preventing the sacrifice of even a small number of animals is more important than attempting to find cures for these diseases.

Another important step is to have scientists recognize that their public information roles must be enormously enhanced. The Foundation for Biomedical Research is already providing some of the needed figures and brochures. Scientists must begin to speak up on television programs and in local interviews to present the case for science. We and our societies must learn how to respond in the era of the 30-second sound bite—not with long and scholarly answers to questions on what is admittedly a complicated subject, but with answers that are short and illuminating. Salient facts, such as that thousands of cats and dogs are killed in research while millions are killed as strays by pounds and shelters and billions of animals are killed for food, should be presented. More scientific societies need public information offices to provide volunteer speakers with information to replace innuendoes with facts. Then more scientists would feel confident in accepting invitations to speak on behalf of research.





The public should be informed or reminded that research on animals also benefits other animals. An image has been created that *Homo sapiens* is ruthlessly exploiting other species in order to improve only his own lot. In fact, a vaccine for rinderpest, a virus that kills millions of cattle slowly and painfully, was developed by animal experiments; the vaccine is now applied by the World Health Organization to millions of cattle in Africa. A vaccine for the deadly feline leukemia was developed through research with cats. A vaccine for anthrax evolved from experiments with sheep. Animal research to help animals is an extremely important story, but it has been obscured by headlines screaming about species exploitation.

A campaign of public information clarifying the real issues and emphasizing the benefits of basic research to both humans and animals, carefully carried out, is urgently needed. The goal should not be to turn around advances made in the care of animals in research, in which constructive humane societies can play a worthy and legitimate role. The goal should be to defeat inappropriate restrictions that may hinder research designed to benefit both animals and humans.—DANIEL E. KOSHLAND, JR.





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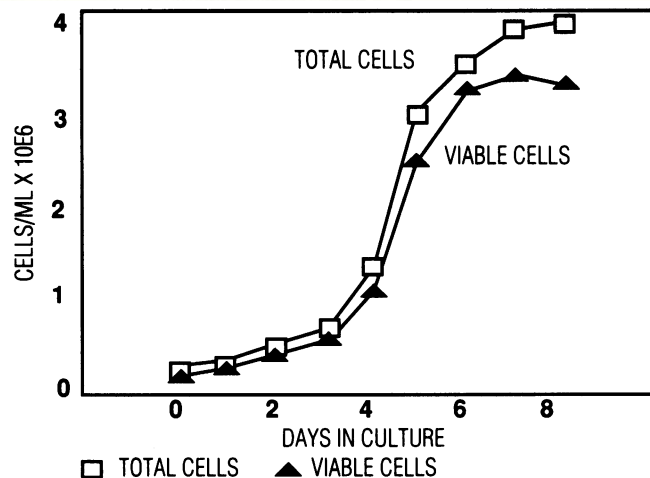
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**FIG.1.** Growth kinetics of Sf-9 cells in an 8L airlift bioreactor using EX-CELL 400™ Medium.

## Letters

### Epidemiologic Investigation

In his article "Scientific standards in epidemiologic studies of the menace of daily life" (2 Dec., p. 1257), Alvan R. Feinstein reviews several axioms of epidemiologic investigation. However, among his examples of objectionable practices he has listed an investigation of some 121,000 American nurses who for many years have supplied data on various aspects of their lives in order to test a number of hypotheses that are of the utmost interest to the public health. Citing one of the findings in this study (1)—that there was an association in these women between ingestion of alcohol and cancer of the breast, Feinstein accuses the investigators (epidemiologists of major stature) of "dredging" up the association, presumably by examining the data for a large number of associations, and then finding one by chance. Nothing could be farther from the truth. The hypothesis associating ingestion of alcohol with carcinoma of the breast has been advanced many times. Feinstein cites abstracts of one case-control study and two small cohort studies in which no association was seen, but neglects to mention that in eight of 12 case control, and four out of four prospective, large cohort studies, distinct associations were found between ingestion of alcohol and carcinoma of the breast. Furthermore, had Feinstein inquired of the investigators, he would have found that a question about alcohol was included in the initial questionnaires prepared in 1980, so that the relation between alcohol and malignancies could be investigated, as clearly stated in the initial grant proposal. Also, as stated in their paper, when the investigators of the Nurses Health Study initially found an association between alcohol and carcinoma of the breast on the basis of the first 2 years of accumulated data, they were concerned lest this association be due to chance alone. Accordingly, they set aside the first 2 years of data and collected the next 2 years of information, analyzing the latter independently; again the association was found. None of the investigators who found this association, which now appears not to be due to chance alone, has claimed a causal relation, but this possibility cannot be excluded (2).

We are forced to suggest that at best, Feinstein may not have reviewed the literature thoroughly. Any questions that he might have raised concerning the conduct of the study would have been answered courteously and promptly. He chose instead to demean the study and the investigators be-

fore he had determined, with the use of his stated criteria of precision, whether his claim was justified.

Large epidemiologic studies are difficult to conduct, and it is easy to become concerned about potential flaws that are quantitatively unimportant. This may have led Feinstein and his colleagues in the past to doubt the validity of a number of conclusions that are now beyond doubt, such as the relation between exogenous estrogens and endometrial carcinoma (3), the relation between diethylstilbestrol and vaginal carcinoma (4), and the relation between aspirin and Reyes syndrome (5).

I believe that the Nurses Health Study, and its investigators, satisfy the most rigorous criteria of scientific accuracy.

EDWARD H. KASS

Channing Laboratory,  
Brigham and Women's Hospital, and  
Harvard Medical School, Boston, MA 02115

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With respect to reserpine and breast cancer, Feinstein states that "in a retrospective attempt to explain the error, one of the original investigators (S.S.) said that the first reserpine-breast cancer association was probably a "statistically significant" artifact due to multiple calculations done during data dredging" (1). (i) The phrase "data dredging" is pejorative; I did not use it. (ii) I referred to multiple comparisons, not multiple calculations—a very different matter, both conceptually and procedurally. (iii) I did not say, or imply, that the finding was an "artifact": I presented preliminary data, since published in full (2), and presumably known to Feinstein (3), showing that the original positive association was likely to be due to chance, "statistical significance" notwithstanding. (iv) Feinstein does not mention several principles that I and others (1) suggested should be applied to multiple comparisons, including, in particular, that no association should be taken seriously unless it can be replicated repeatedly, independently, by means of other methods, and in greater detail.

The association between alcohol and breast cancer was not *found* in the studies (4) to which Feinstein refers, but was *confirmed* in them. Earlier, Williams and Horm (5) observed an association (and a dose-response relation) with lifetime alcohol con-

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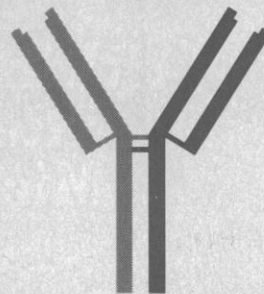
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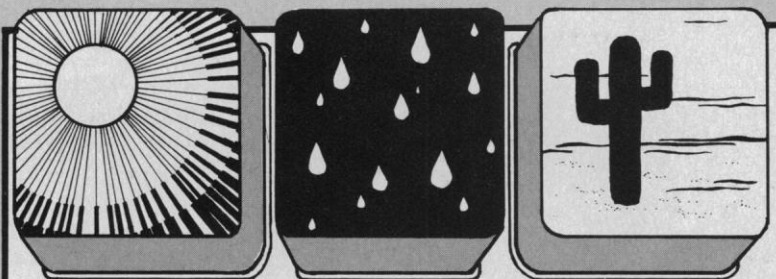
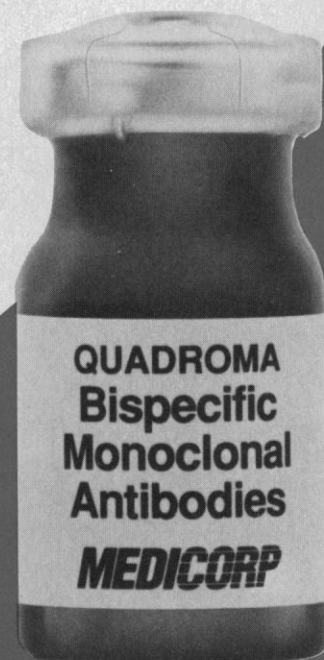
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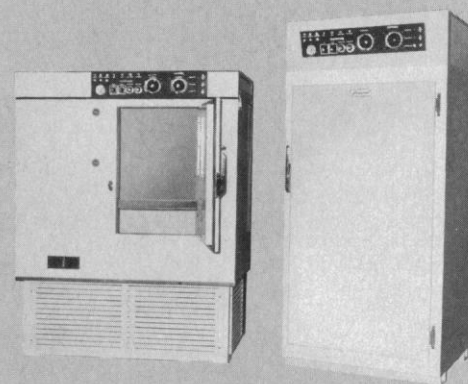
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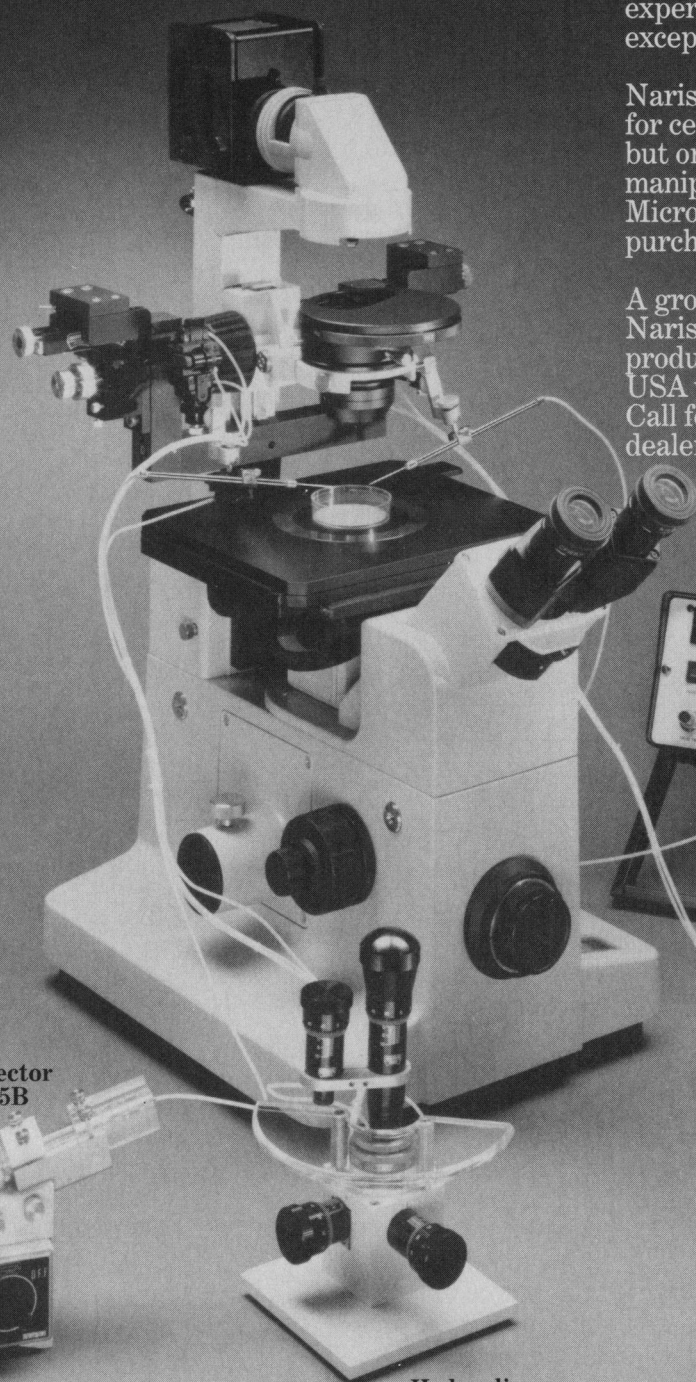
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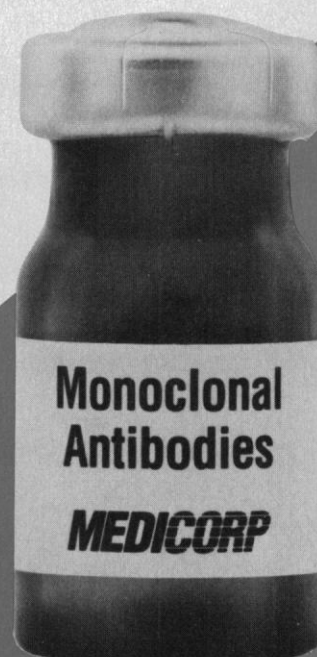


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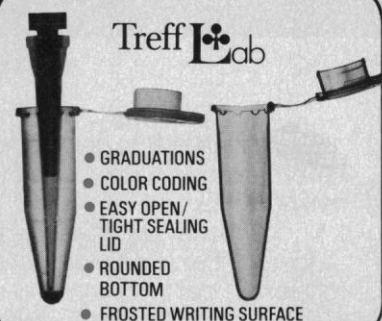
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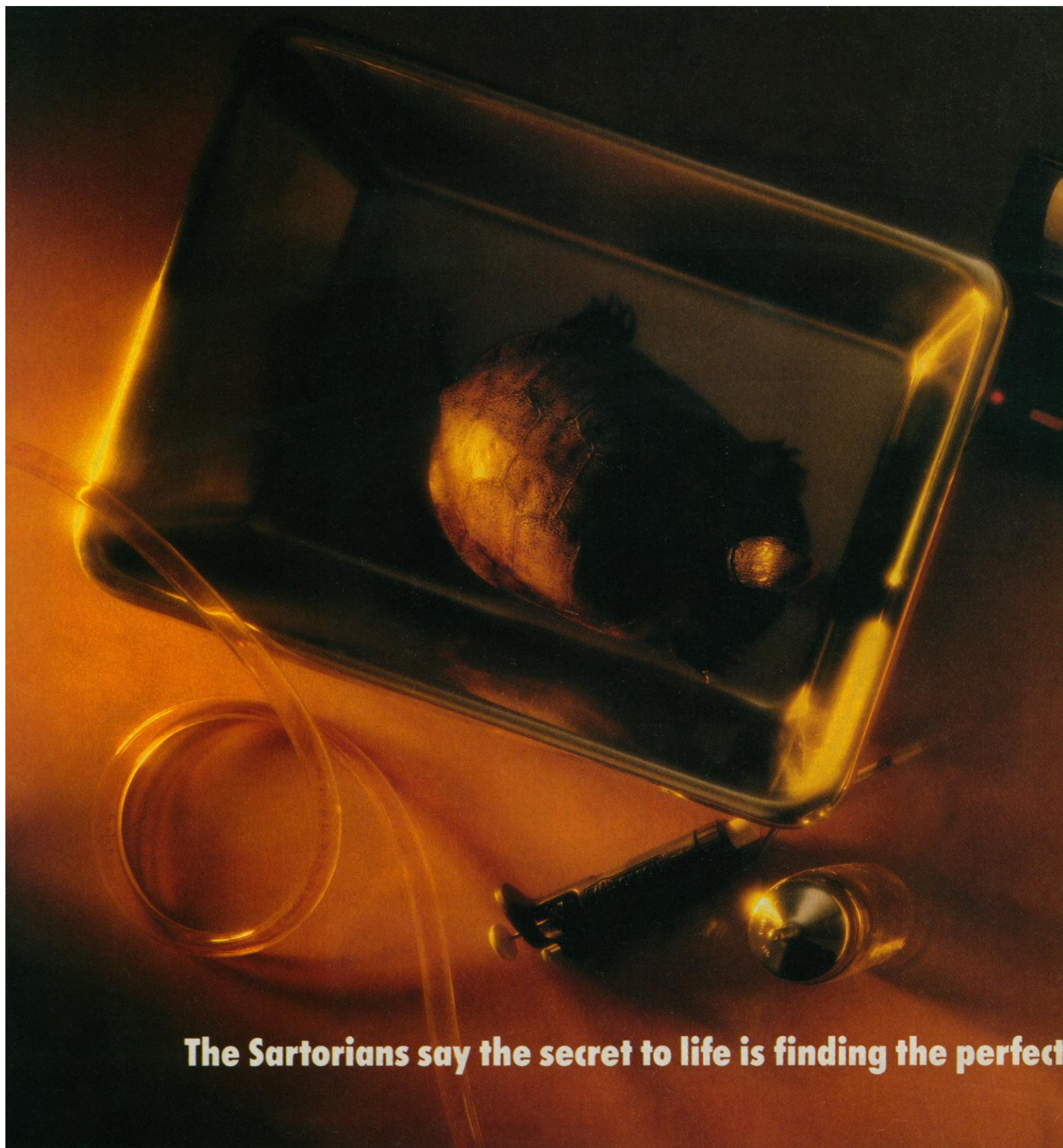
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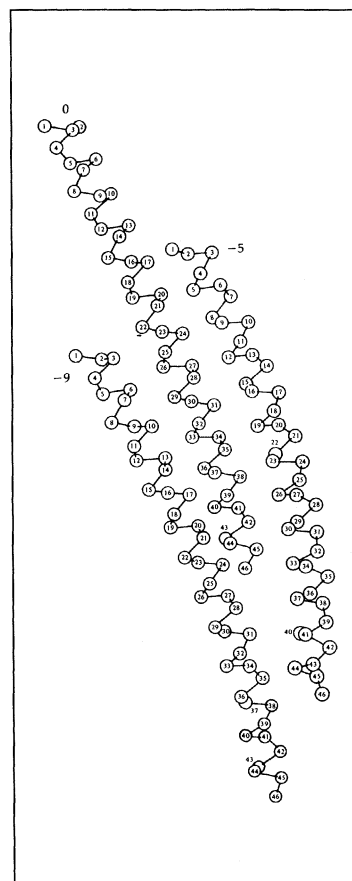
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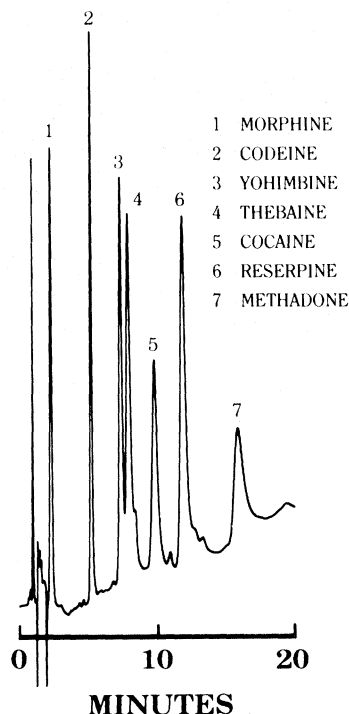


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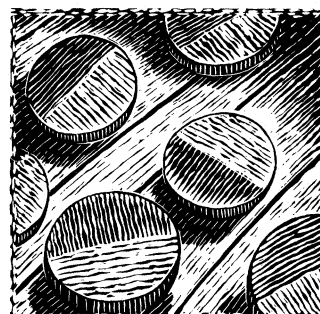
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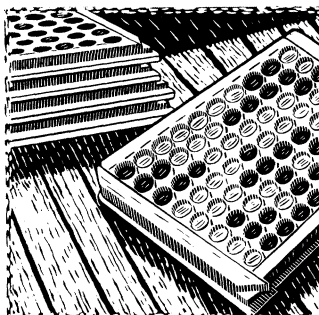
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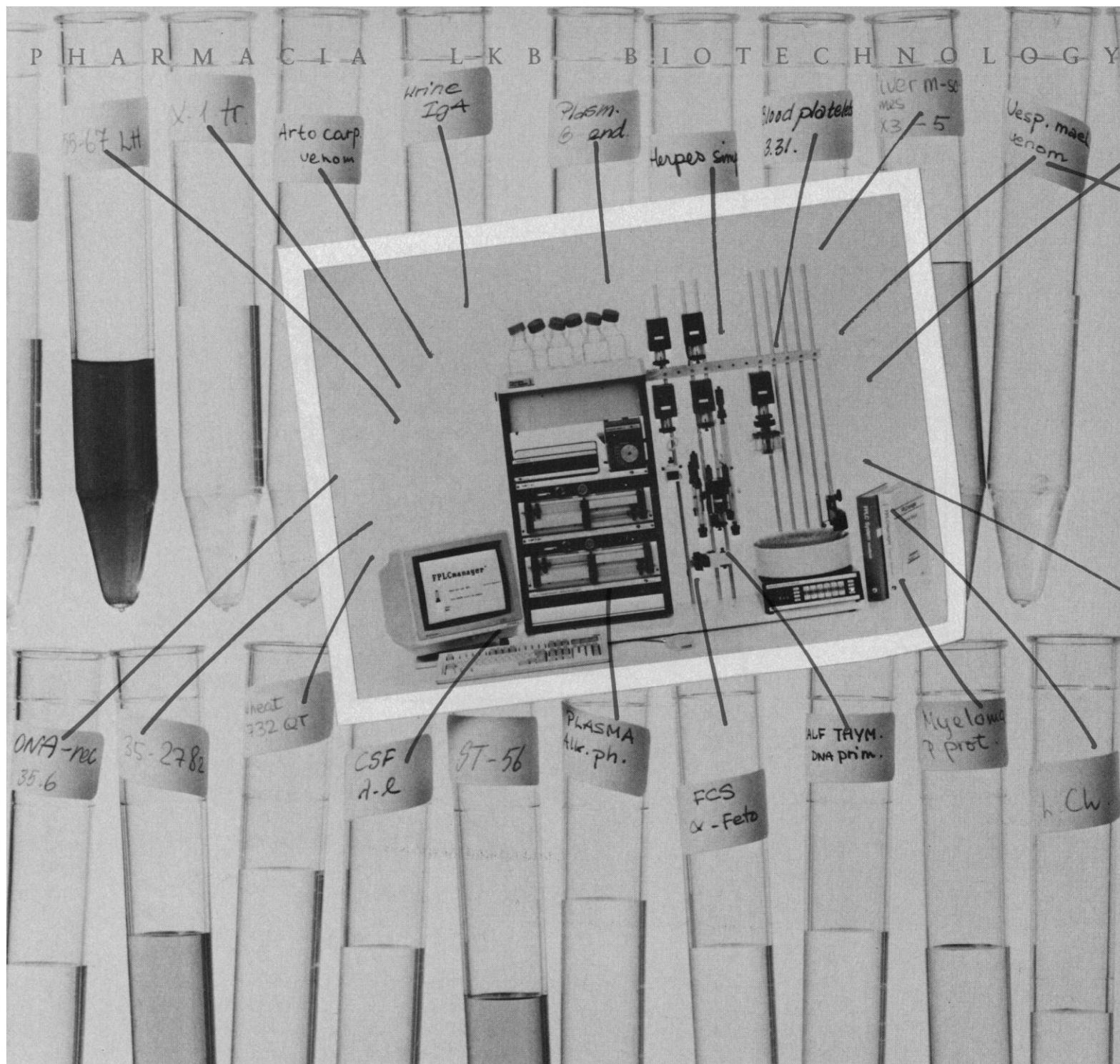
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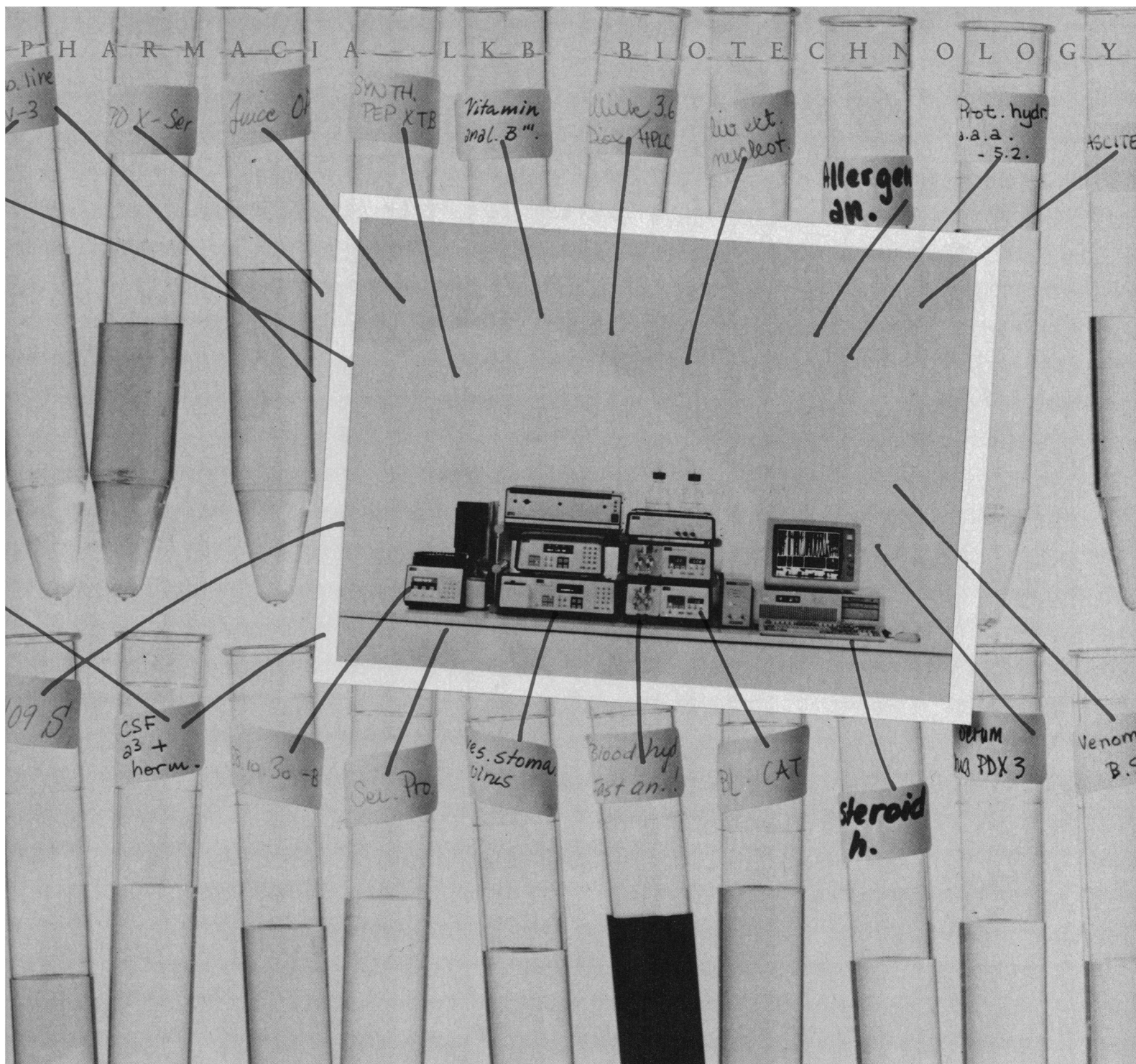
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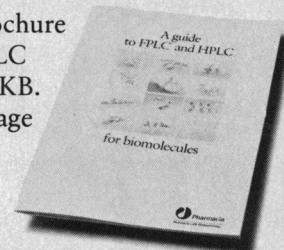
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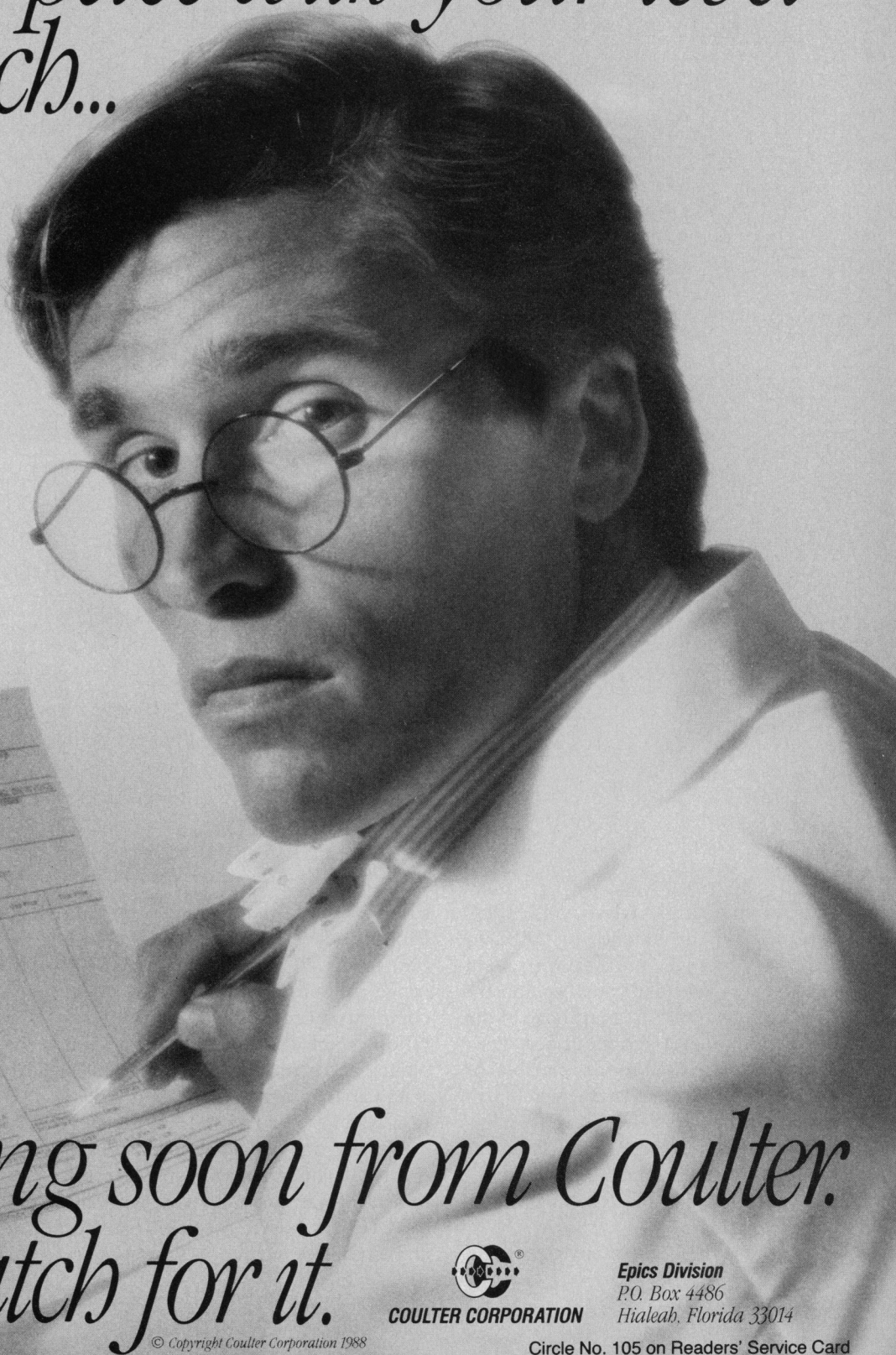
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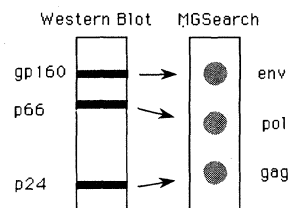
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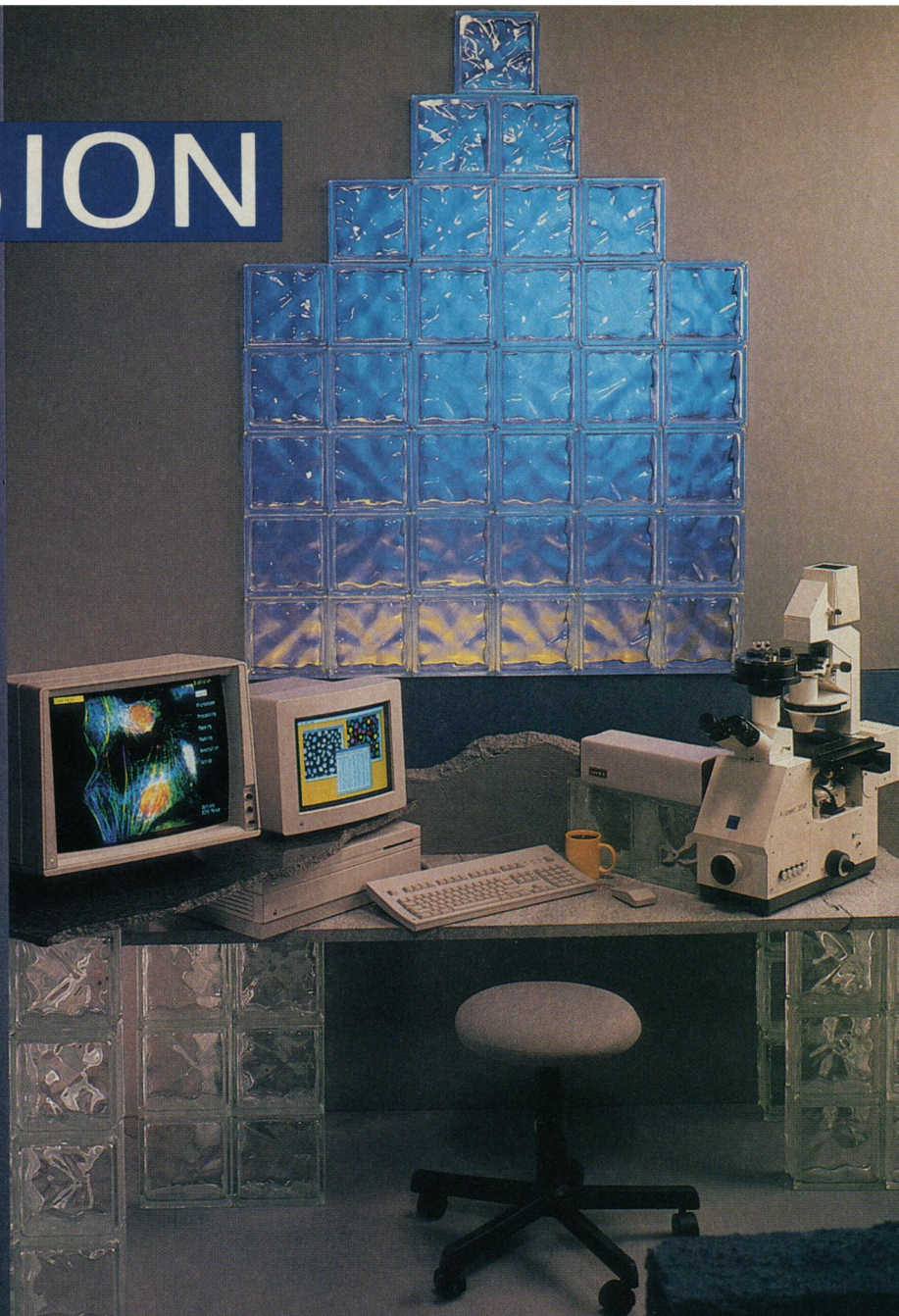
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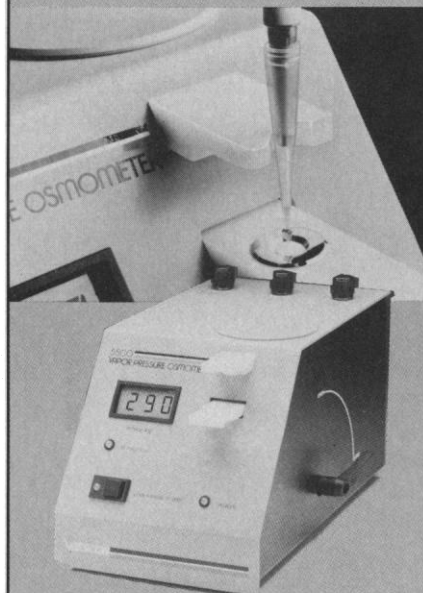
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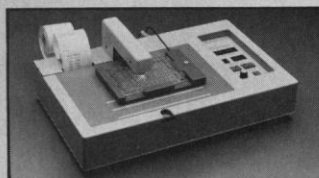
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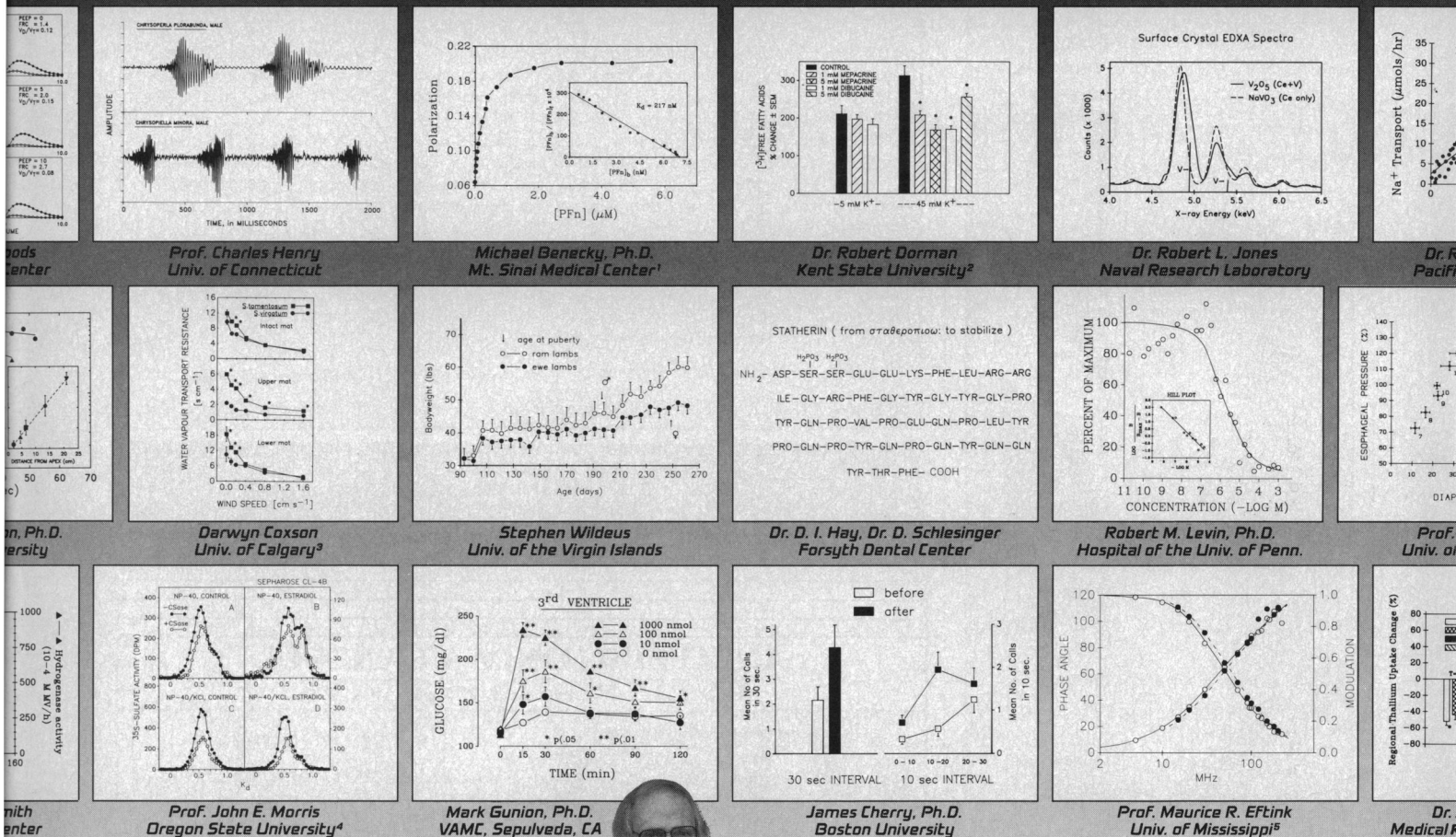
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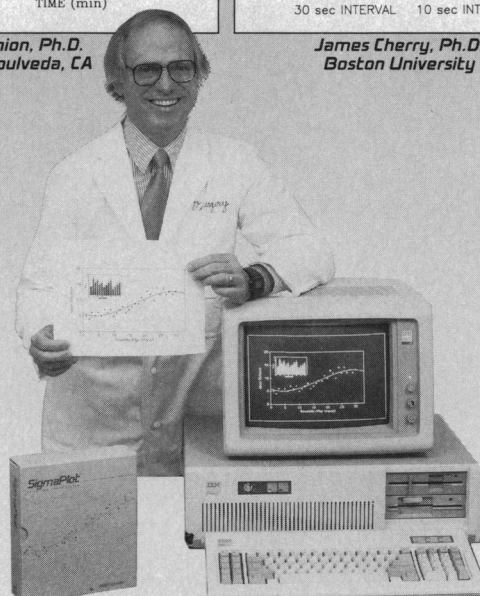
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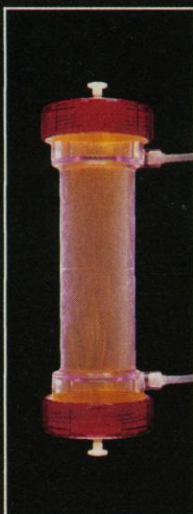
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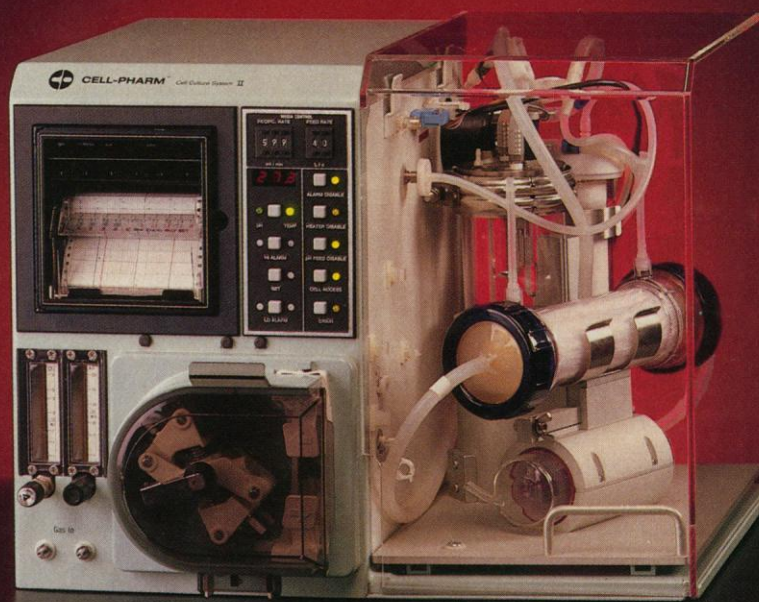
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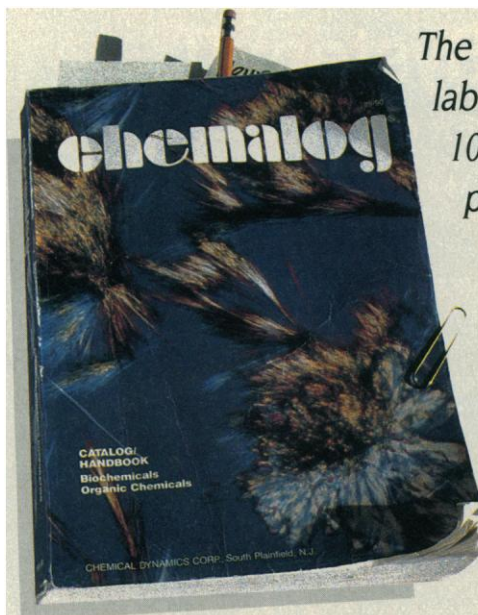
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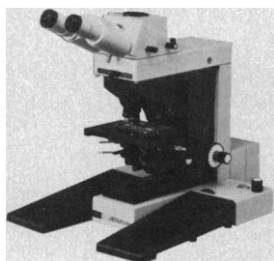
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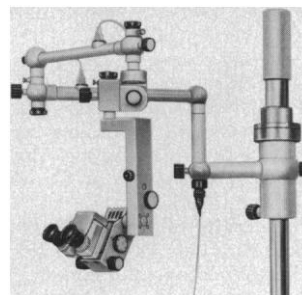
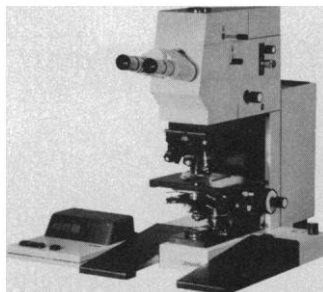
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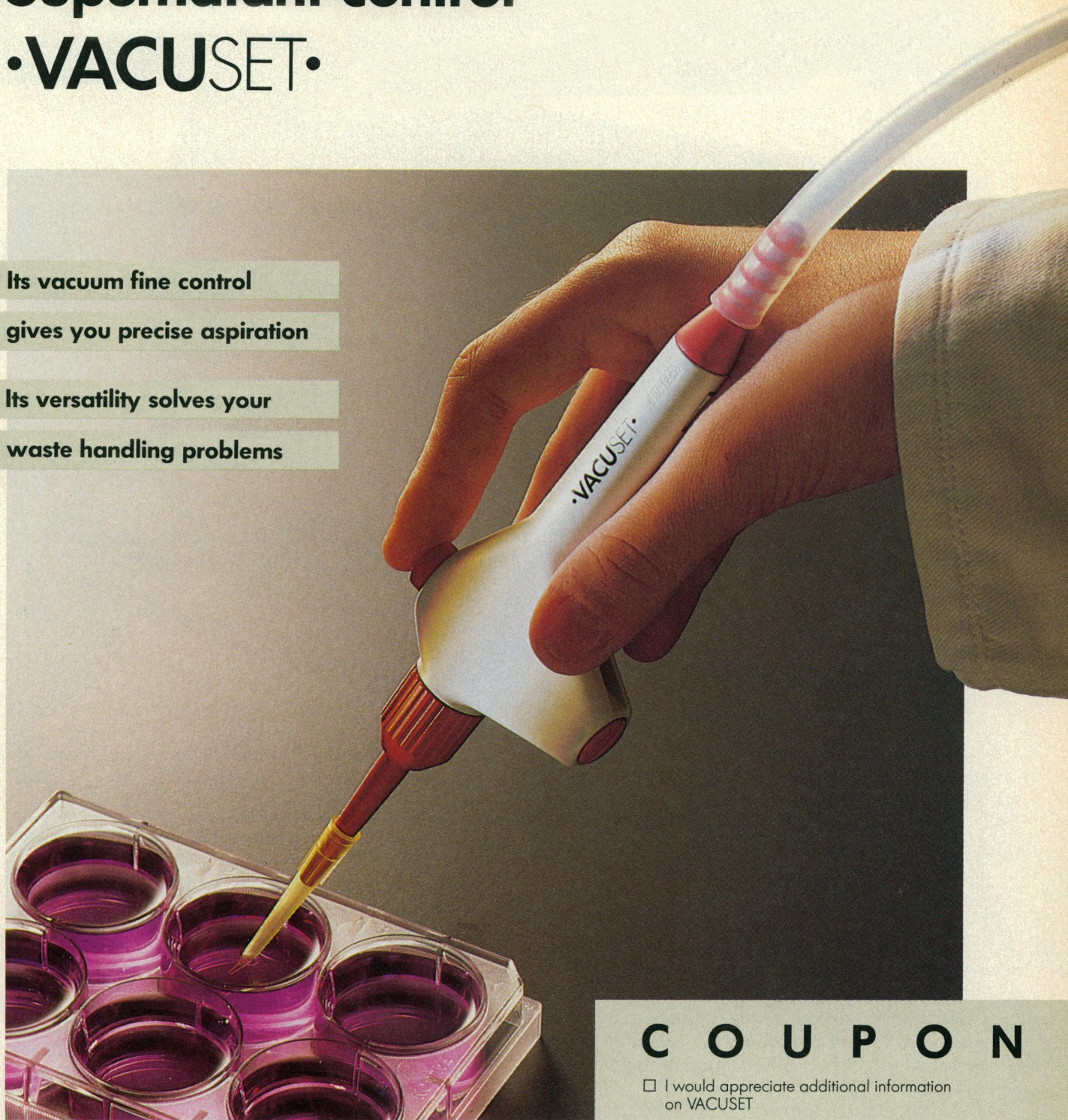
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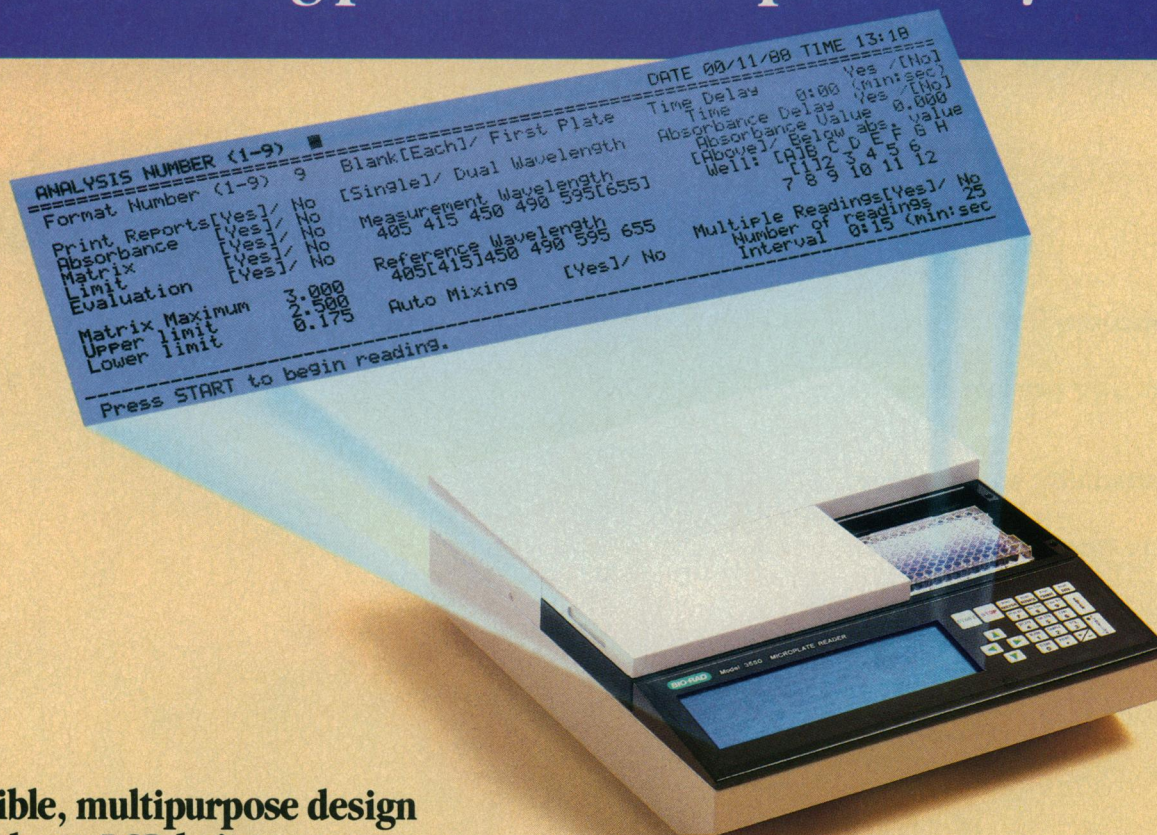
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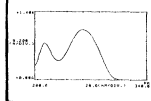
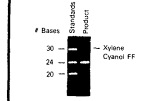
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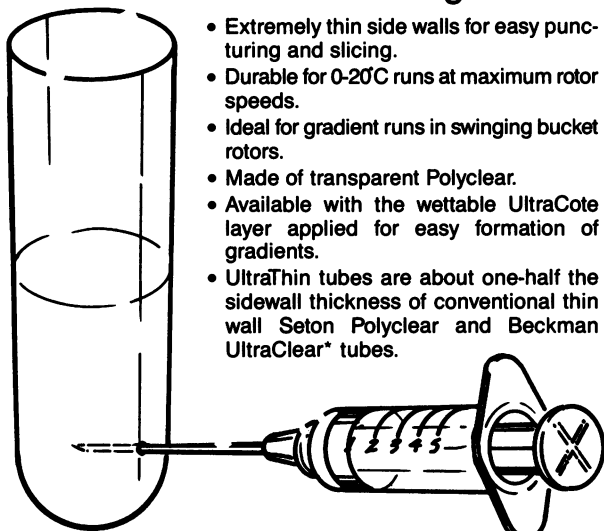
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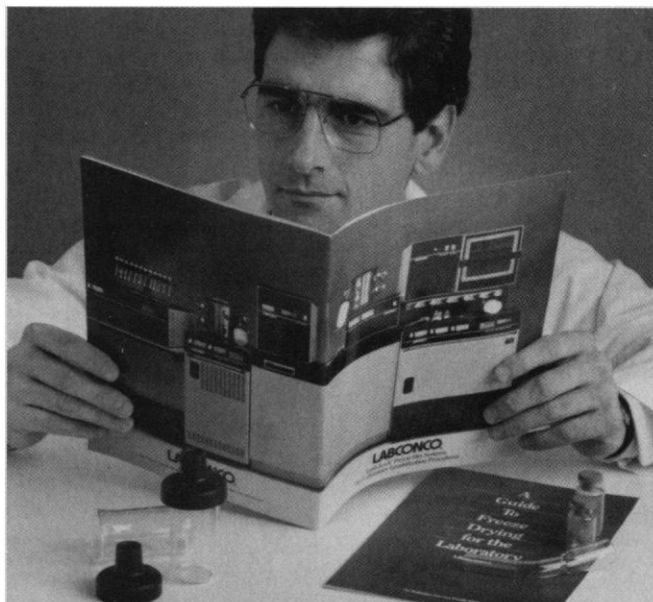
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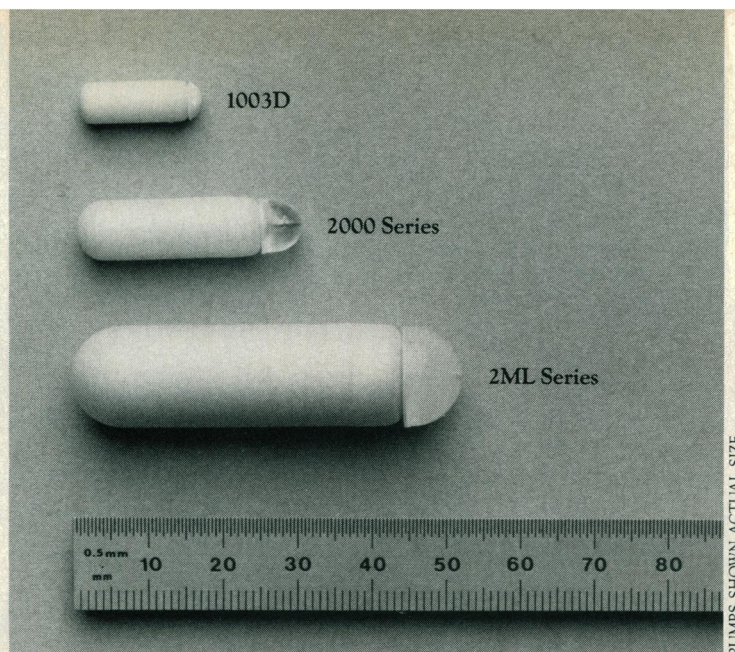
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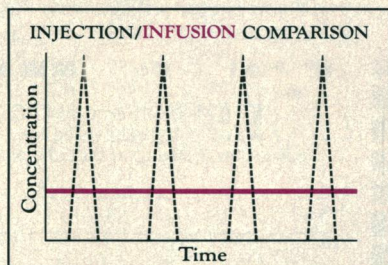


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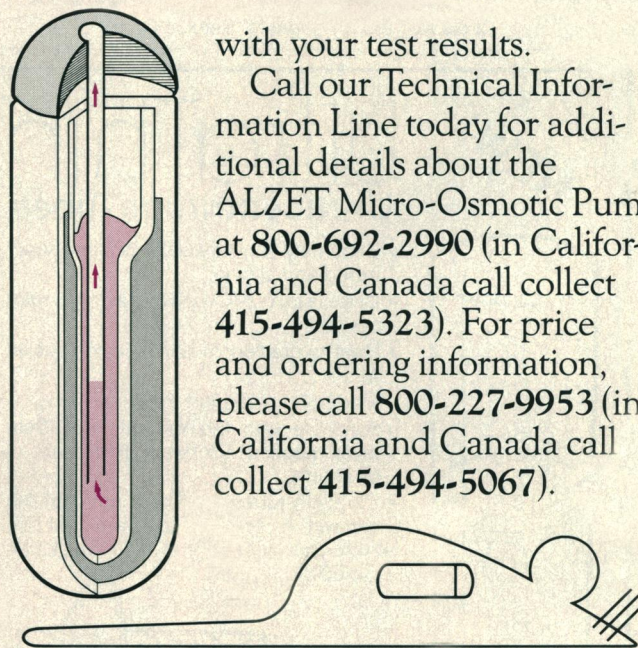
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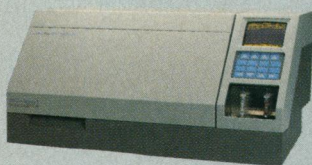
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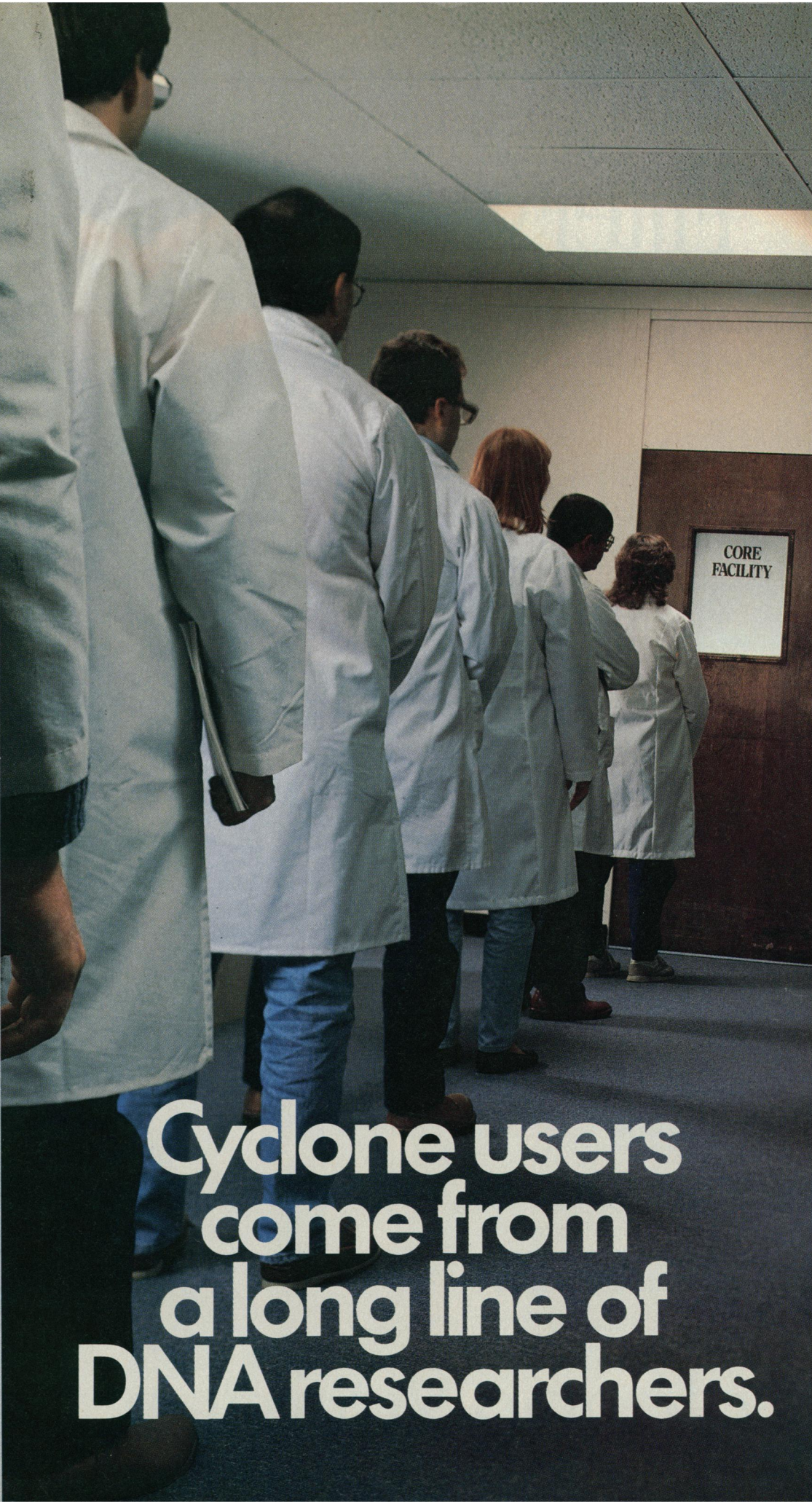
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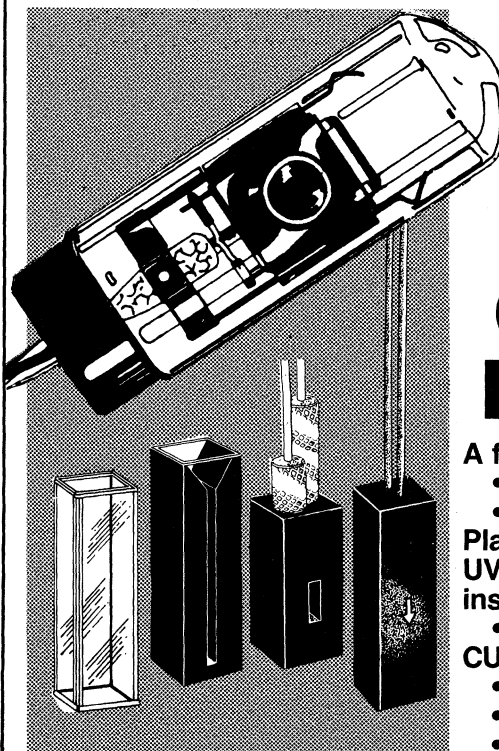
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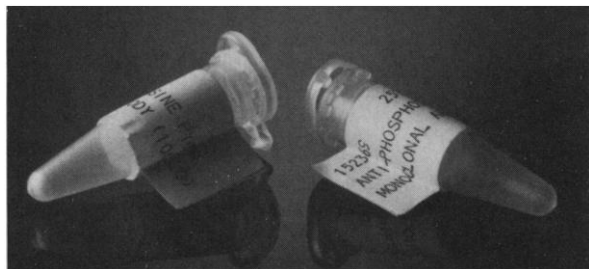
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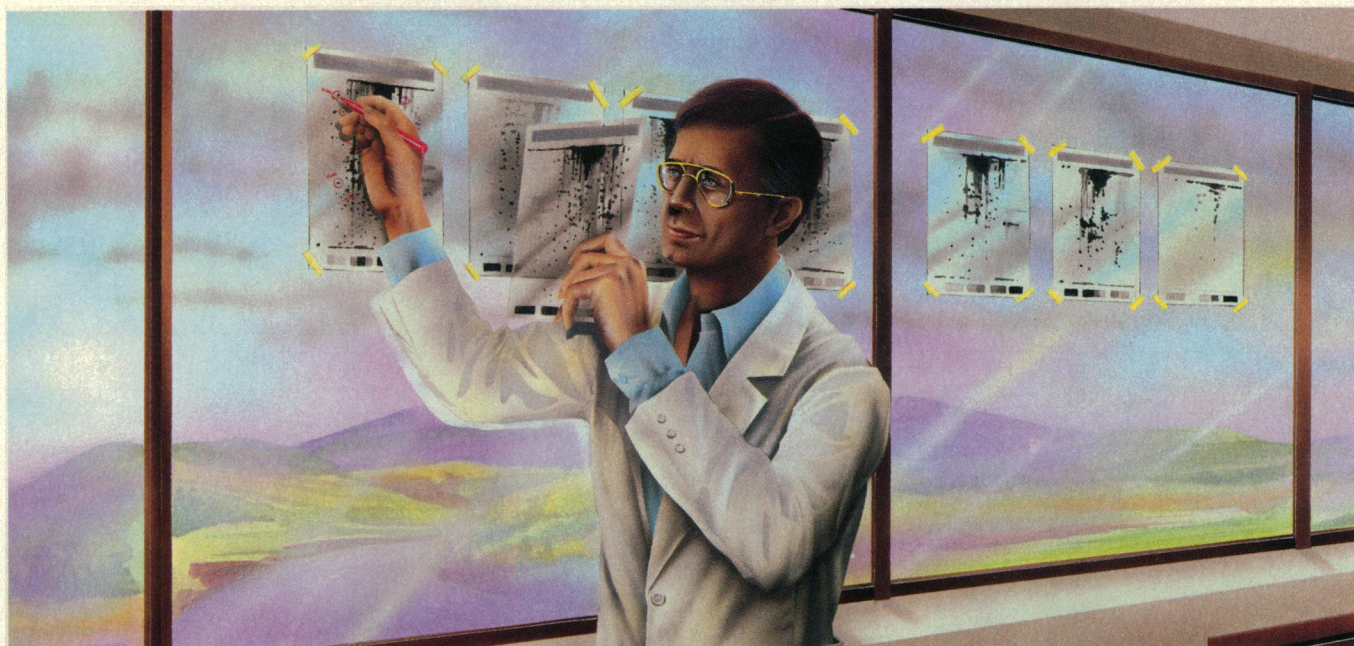
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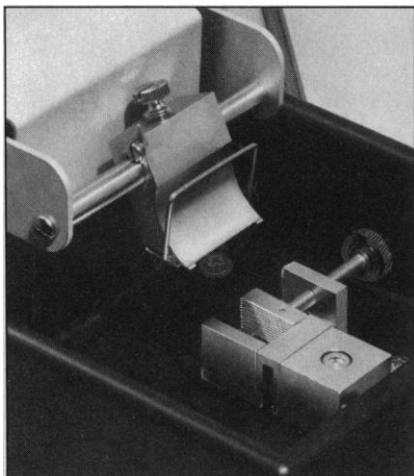
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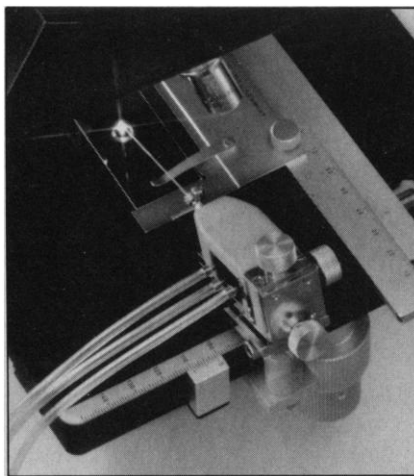
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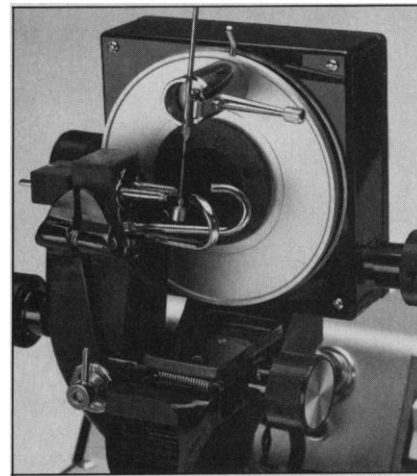
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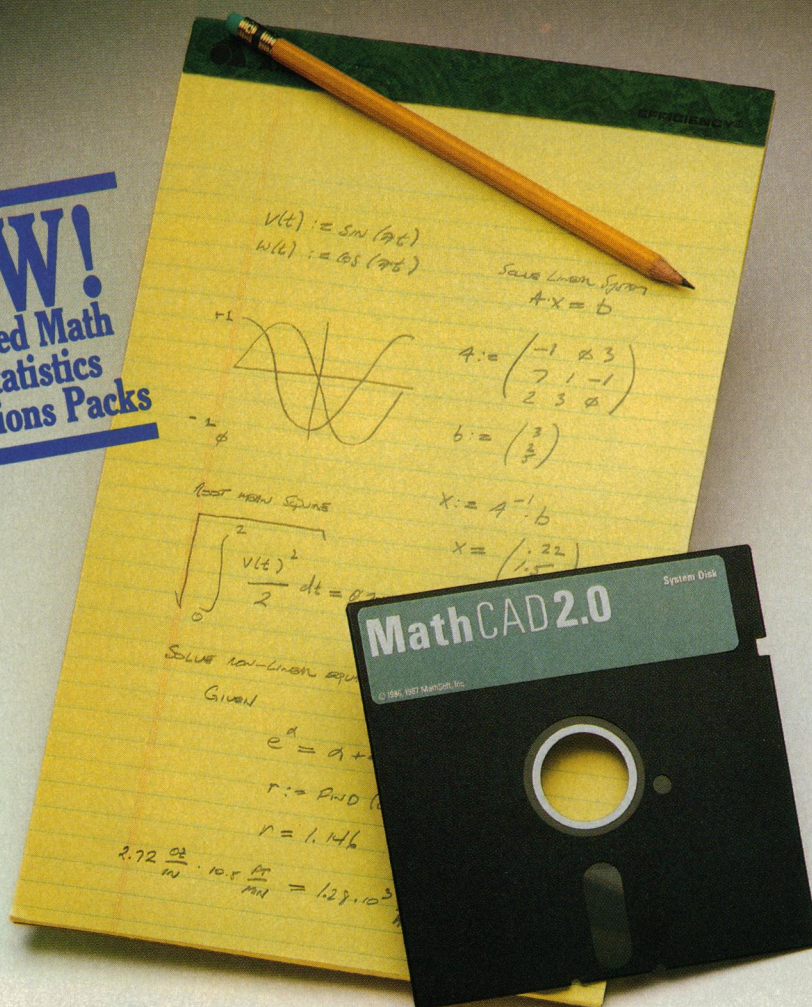
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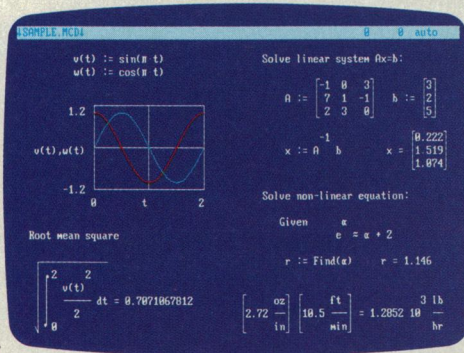
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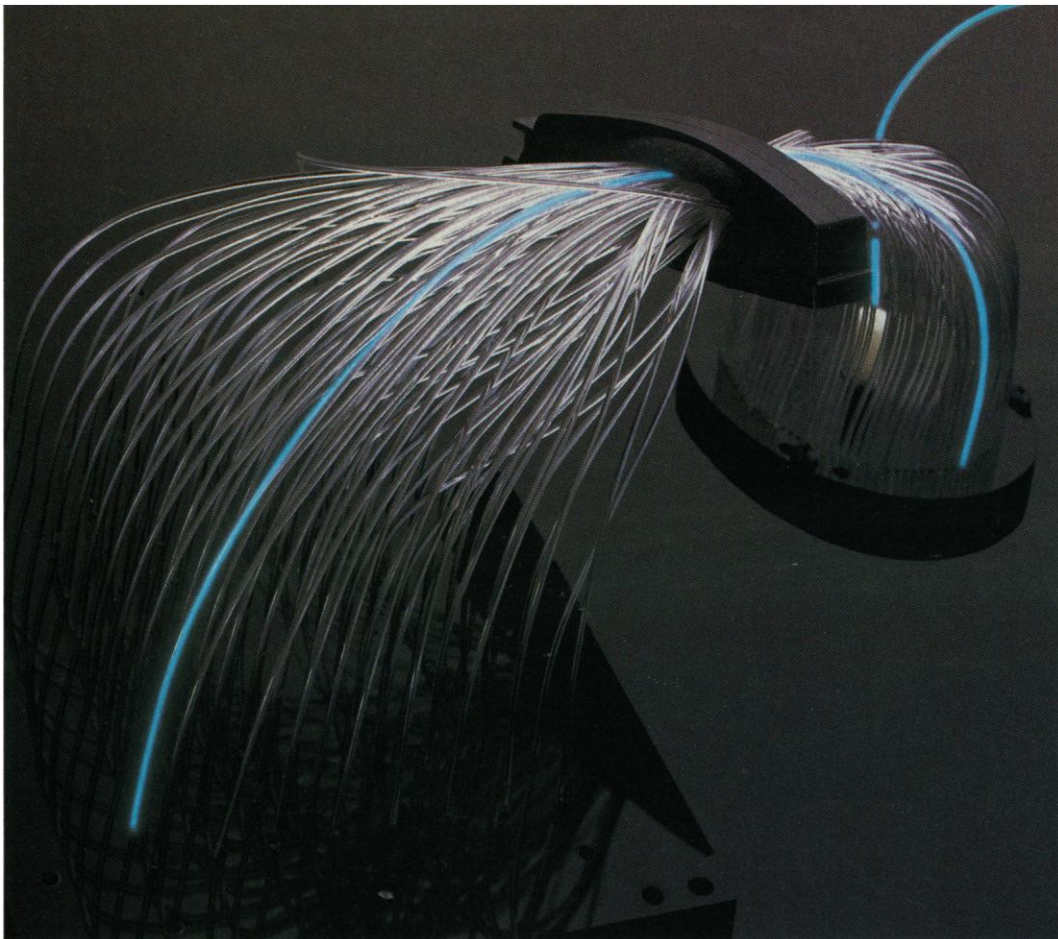
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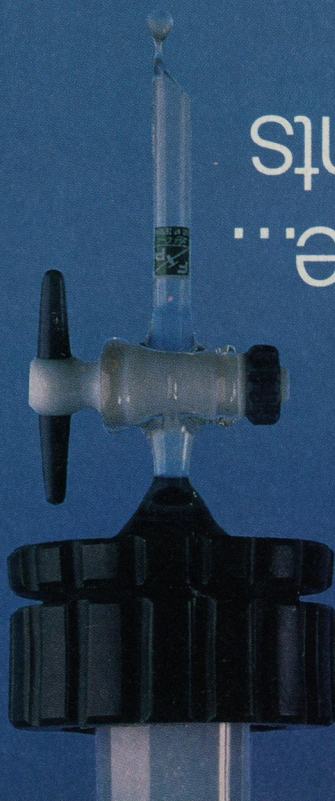
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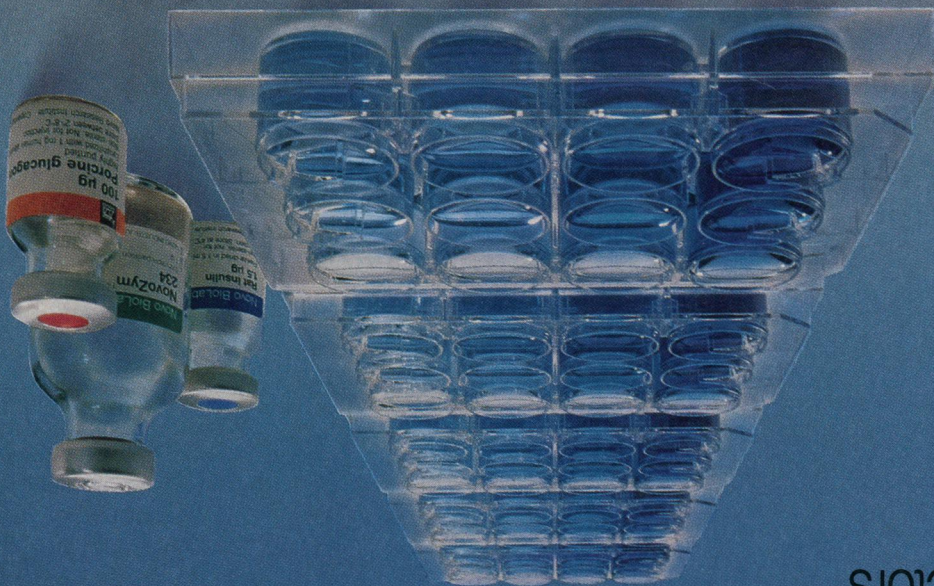
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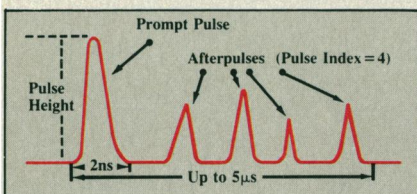
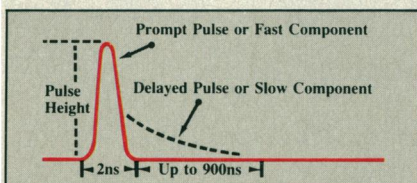
In the past, liquid scintillation counting used traditional technology to overcome background noise. Now, there's a new, more sensitive technology – TR-LSC or Time-Resolved Liquid Scintillation Counting – that reduces background noise by an additional 30%-40%, and more. This new patented technology is available only in Packard's Tri-Carb® liquid scintillation analyzers.

Originally developed for extremely low level counting, TR-LSC technology has now been applied to a broad range of applications. While these don't always require high sensitivity, additional benefits have been realized. By increasing sensitivity, TR-LSC reduces sample and cocktail consumption while shortening the time required for accurate counts. The benefits? Lower cocktail costs, lower disposal costs, and increased throughput.

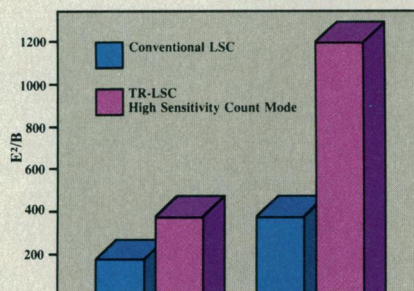
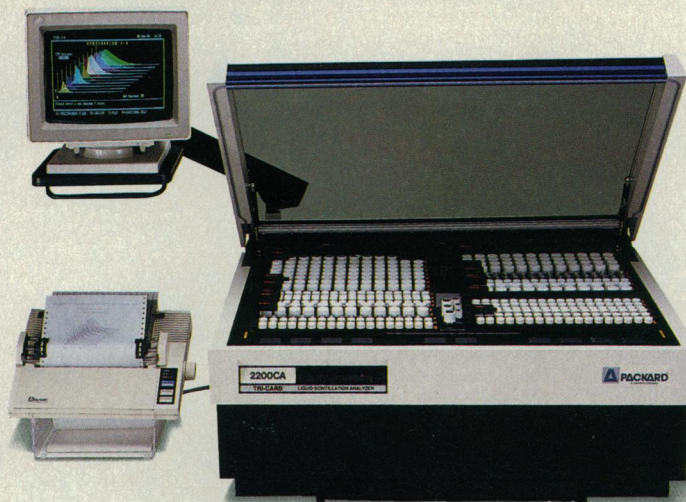
## How TR-LSC is superior to older technology

Traditional counters are based on two-dimensional pulse analysis: pulse height and pulse counts. They provide a level of sensitivity that's merely adequate for most applications.

Patented TR-LSC adds a third dimension to pulse analysis: a pulse index that measures over time the afterpulses associated with background. In doing so, TR-LSC clearly distinguishes between beta pulses and background pulses. By identifying, and reducing, background noise, TR-LSC provides a great level of sensitivity (see chart comparing  $E^2/B$  values) and more accurate counts.



The typical beta scintillation pulse (top) is very fast and may be followed by a delayed component. The typical background pulse is followed by a series of afterpulses. Patented TR-LSC distinguishes between the two.



Typical  $E^2/B$  values for  $^3\text{H}$  and  $^{14}\text{C}$  using traditional and TR-LSC counting.

## Achieve accurate counts on samples as small as 25 µL

Traditional technology limits sensitivity. The improved sensitivity of TR-LSC, however, allows you to achieve accurate DPM results for single and dual label samples in volumes as small as 25 µL. That can add up to substantial savings in sample and cocktail costs.

## Slash radioactive waste disposal costs

Counting smaller samples will also reduce radioactive liquid disposal costs, which can be \$500 per drum, or higher. While you may not pay this cost directly, your operating budget could be affected. With TR-LSC you can cut operating costs while reducing environmental hazards.

## Increase sample throughput by over 80%

Just as TR-LSC reduces the volume of sample and cocktail required for accurate counting, it also reduces the time required for an accurate count. By cutting background in half, high sensitivity TR-LSC lets you count nearly twice the number of vials of a 250-DPM sample, in the time it would

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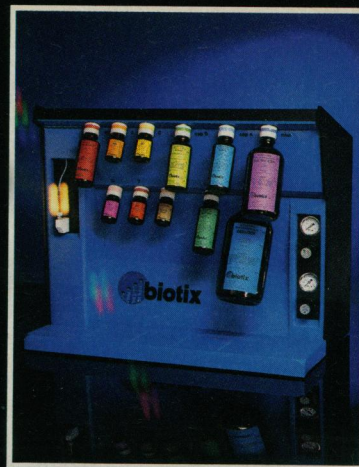
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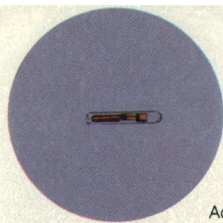
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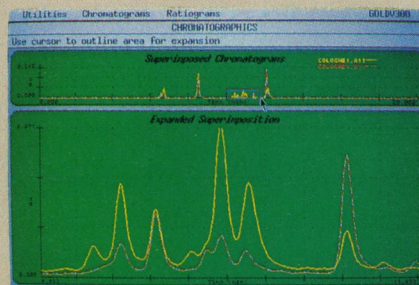
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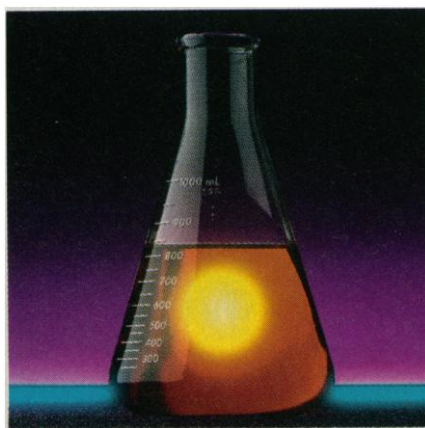
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## The Study of Memory

**Remembering Reconsidered.** Ecological and Traditional Approaches to the Study of Memory. ULRIC NEISSER and EUGENE WINOGRAD, Eds. Cambridge University Press, New York, 1988. x, 390 pp., illus. \$44.50. Emory Symposia in Cognition, vol. 2. From a conference, Atlanta, GA, Oct. 1985.

Most contemporary psychologists would agree that a proper understanding of human memory will contribute greatly to, and may even constitute a necessary condition of, progress in cognitive science. But there would likely be less agreement concerning the most efficient route to attaining such an understanding. In 1885, a German scientist named Hermann Ebbinghaus published a pioneering study that laid out one approach to a scientific psychology of memory. He reported a series of tightly controlled laboratory experiments on learning and remembering of artificial materials that were carefully constructed to minimize possible bias contributed by pre-existing, real-world knowledge. Although Ebbinghaus's studies remain unique in the psychological literature—he served as his own (and only) experimental subject, memorizing thousands of nonsense syllables—his commitment to controlled laboratory experimentation has exerted a profound influence on the field since the publication of the groundbreaking 1885 monograph.

The Ebbinghausian approach to the study of memory has not been without its critics. The eminent French psychologist Pierre Janet expressed skepticism about it in the early years of the 20th century, and the British psychologist Sir Frederic Bartlett launched a more extensive and better-known attack in 1932. But the most recent and influential challenge was advanced by in the late 1970s by Ulric Neisser. Neisser expressed disappointment with the lack of progress made by laboratory studies of memory and chided psychologists for almost entirely ignoring real-world manifestations of memory in favor of a narrow focus on laboratory study of artificial phenomena. According to Neisser, the experimental study of memory lacked ecological validity and had suffered for it. Neisser urged psychologists to summon the courage to step outside the confines of the laboratory and adopt an ecological approach concerned with functionally important, naturally occurring memory phenomena. During the past decade, a growing number of cognitive psychologists have heeded Neisser's call.

In *Remembering Reconsidered*, Neisser and Eugene Winograd have assembled a collection of papers that explore various aspects of

the ecological approach to memory. One immediately striking feature of the book is that it strikes a conciliatory tone toward the laboratory approach. Whereas one could have easily interpreted Neisser's previous writings as suggesting that the laboratory approach ought to be abandoned altogether, the subtitle of the present volume—"Ecological and Traditional Approaches to the Study of Memory"—implicitly acknowledges the laboratory approach's "right to exist." Moreover, both of the editors develop this theme explicitly in their contributions. Part of the reason for this, no doubt, is that laboratory-oriented studies of memory have flourished during the past decade, producing a variety of important data and novel theoretical frameworks.

The contributions to *Remembering Reconsidered* are of uniformly high quality and contain a wealth of new facts about manifestations of memory in various real-world contexts; they also provide provocative discussions of methodological, conceptual, and theoretical issues. Consider just a few highlights. William Brewer describes an ingenious method for investigating people's memory of naturally occurring, everyday "happenings" that would no doubt have pleased Ebbinghaus himself. Brewer

equipped participants in his study with electronic beepers that were programmed to sound at random intervals; subjects were instructed to record where they were, what they were thinking, and other aspects of what was happening when the beepers sounded. Brewer then collected the protocols and tested subjects' memory at various times after the recorded events by providing them with different aspects of the events as retrieval cues. Brewer's data revealed, among other things, the crucial importance of spatial location in memory for naturally occurring everyday episodes. Wanda Wallace and David Rubin report a study of memory for a folk ballad—"The Wreck of the Old 97"—in which they were able to analyze systematically various transformations in the structure and content of the ballad made by five folk singers, as well as by undergraduates who learned the ballad. They found that alterations in reproduction were related to internal constraints in the structure of the ballad. Katherine Nelson summarizes important developmental studies in which she analyzed naturally occurring pre-sleep "monologues" of a two-to-three-year-old child that contained frequent reference to past events. Nelson observed striking evidence for long-term recall of single

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