SCIENCE

16 July 1976

Volume 193, No. 4249

AMERICAN ASSOCIATION FOR THE ADVANCEMENT OF SCIENCE

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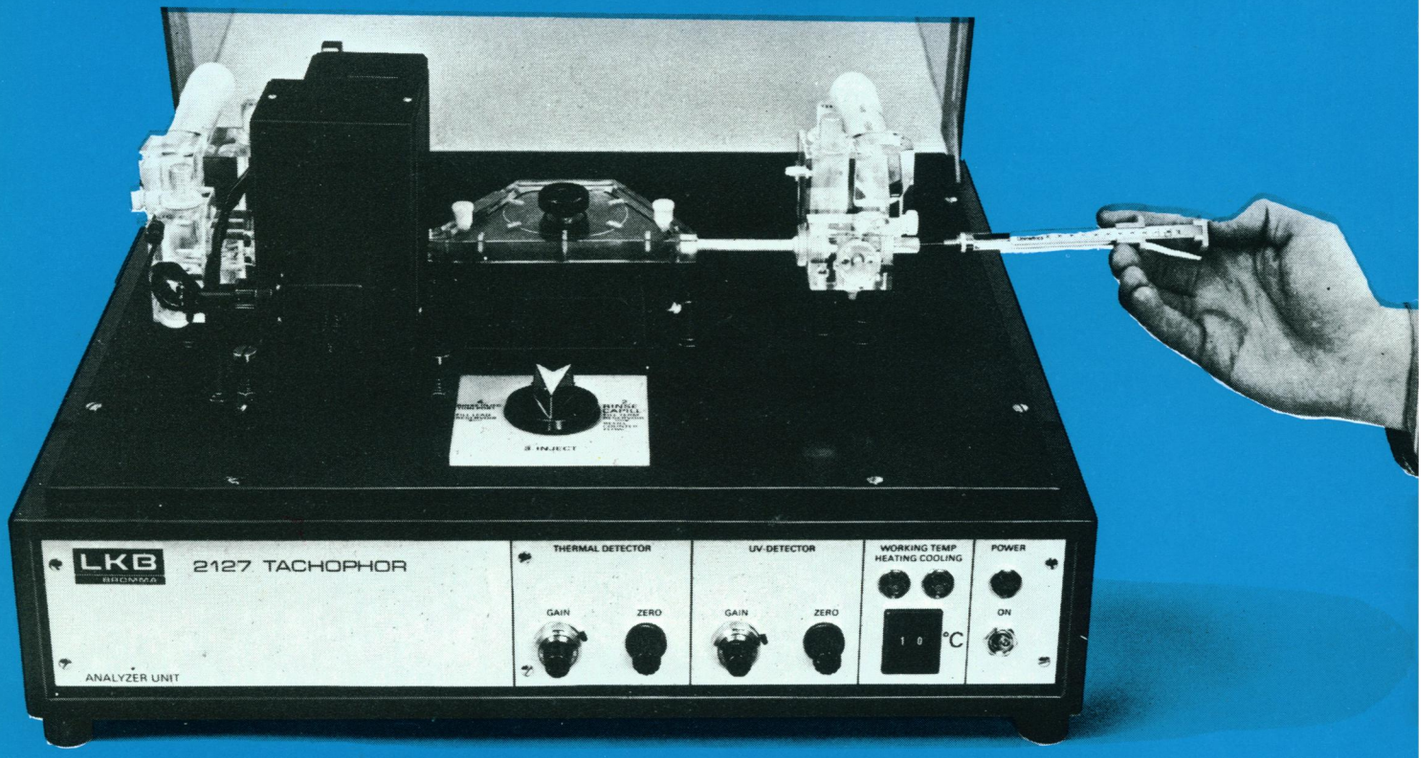

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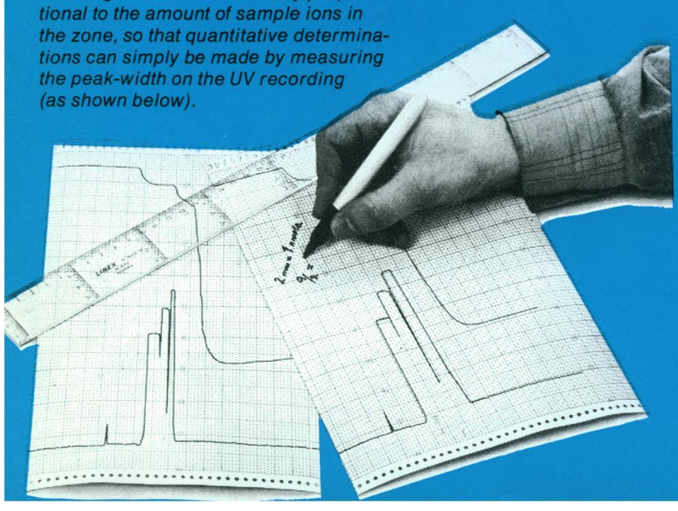

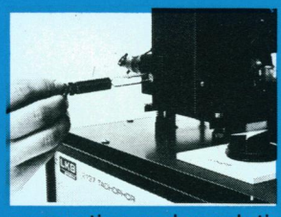

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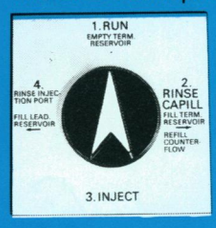

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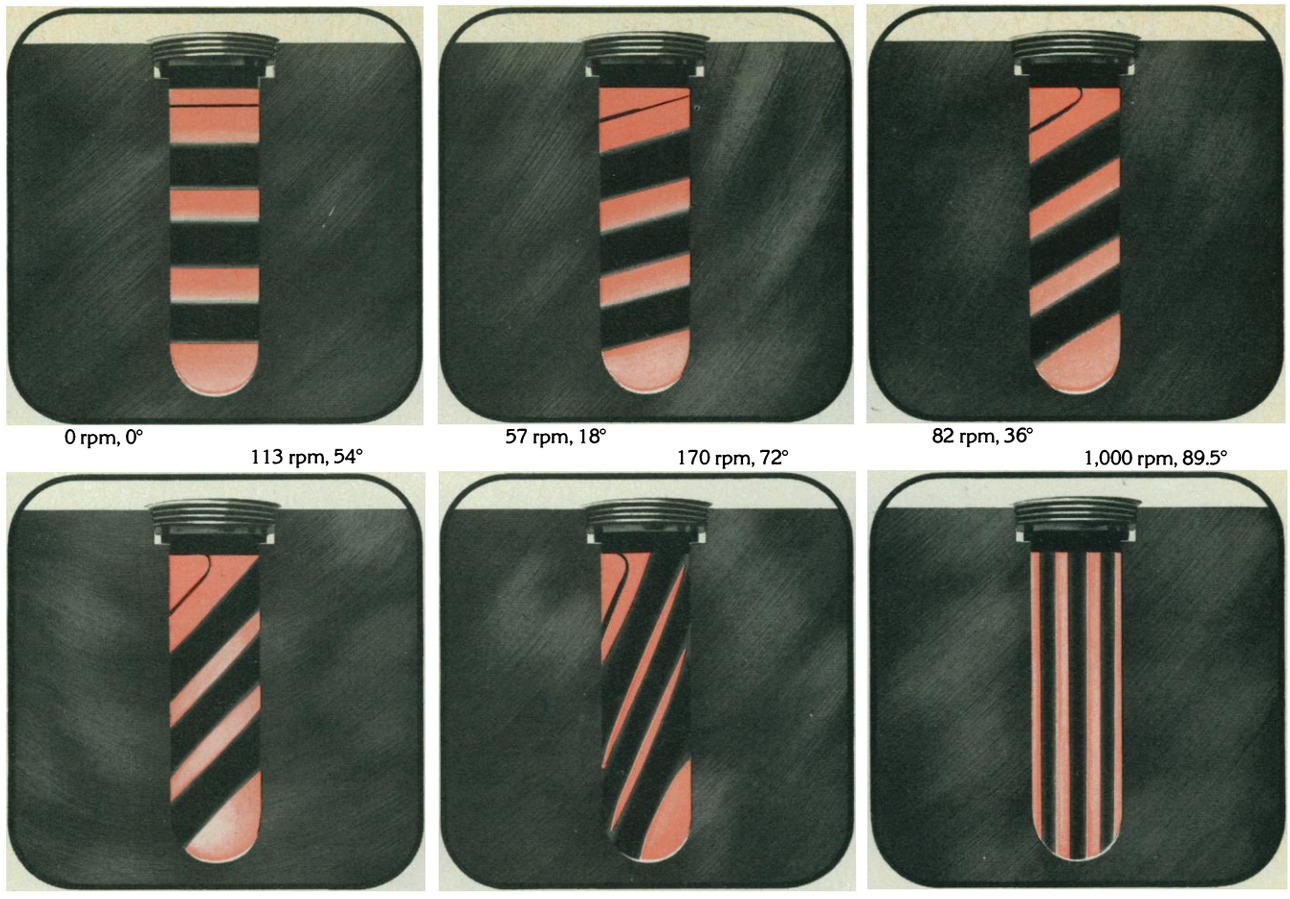

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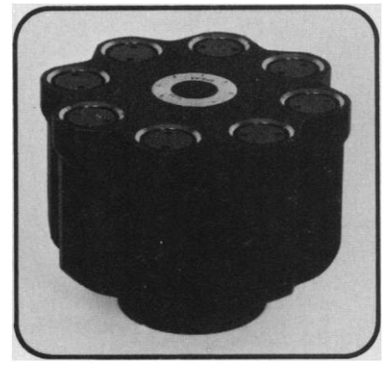

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16 July 1976

Volume 193, No. 4249

SCIENCE

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COVER

Coloration of gibbons. Pileated and lar gibbons have white hands and feet. Concolor gibbons shown are from Laos; males farther east are entirely black. Borneo is divided between two species of entirely different voice but having similar polymorphic coloration in endless variety. Arrows connect taxa with the same female song. See page 235. [Painting by Joe T. Marshall, Jr., U.S. Army Medical Component, SEATO]

NEW
HEM-O-SCAN™
Oxygen Dissociation Analyzer

Produces a continuous, complete hemoglobin-oxygen equilibrium curve in approximately 20 minutes from only two microliters of whole blood or hemolysate.

The Hem-O-Scan analyzer offers a major advance in techniques for the analysis of the oxygen carrying capacity of hemoglobin. It provides a significant time saving, and offers simplicity of operation.

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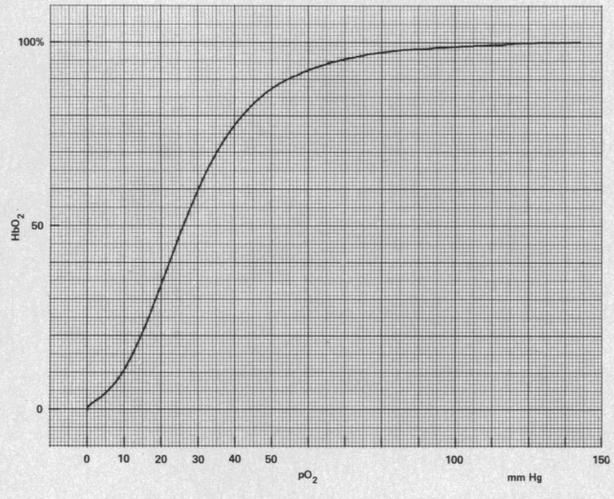
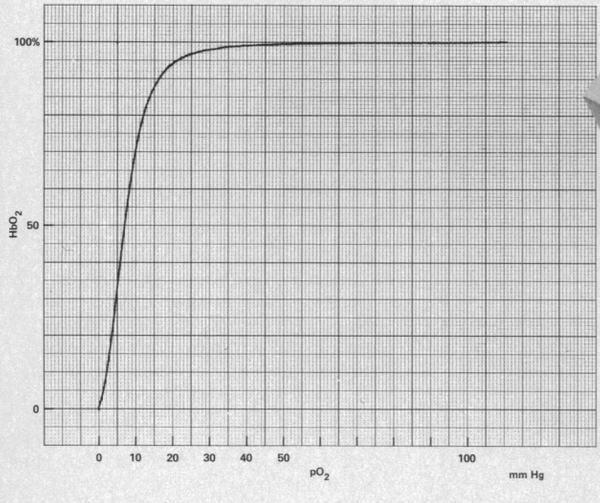
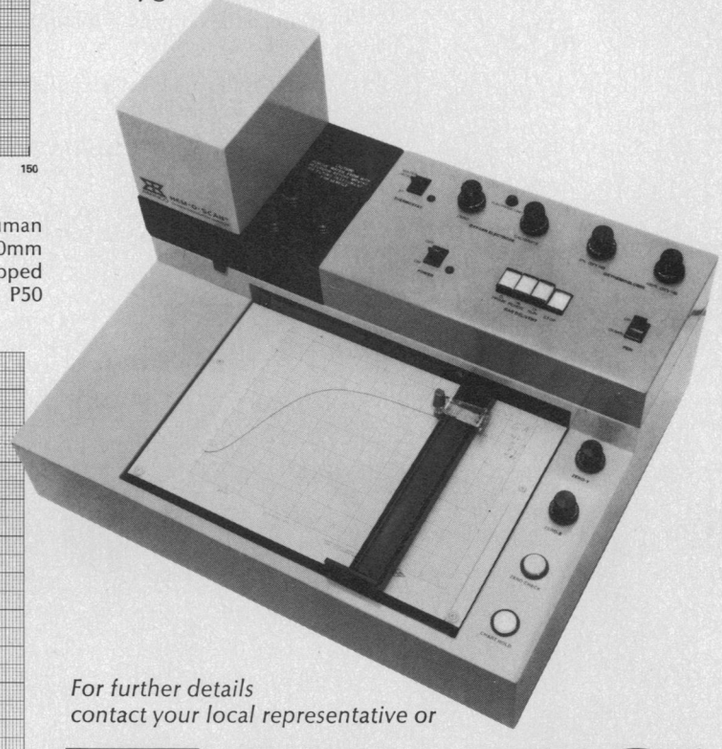


Above: Hemoglobin-oxygen equilibrium curve of normal human blood at 37°C and pCO_2 of 40mm Hg showing a P50 = 26.0mm Hg. Below: Hemoglobin-oxygen equilibrium curve of stripped hemolysate in bis-Tris buffer at 25°C pH = 7.4, showing a P50 = 7.0mm Hg.





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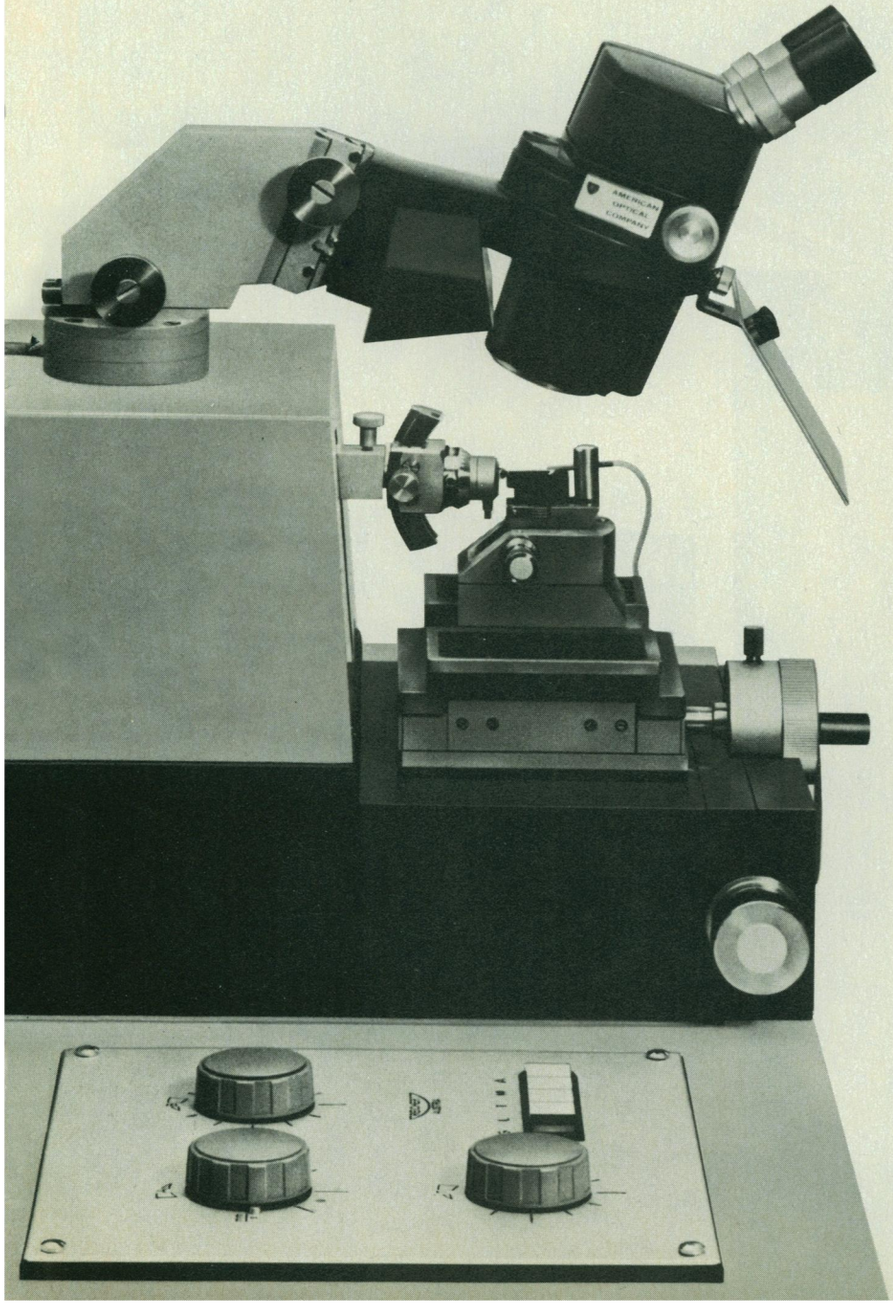

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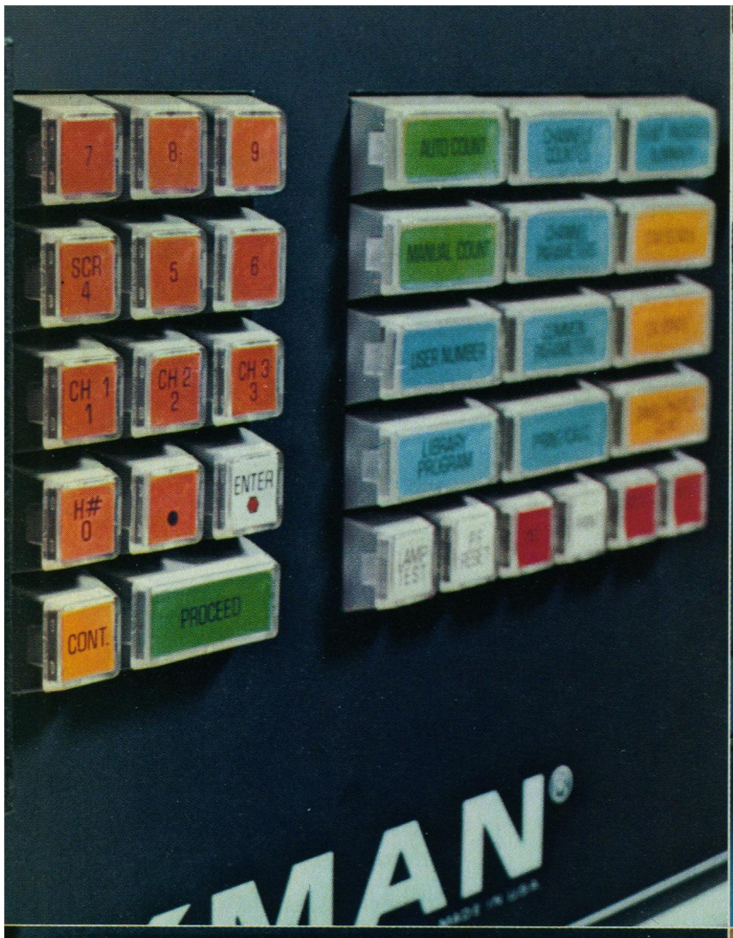
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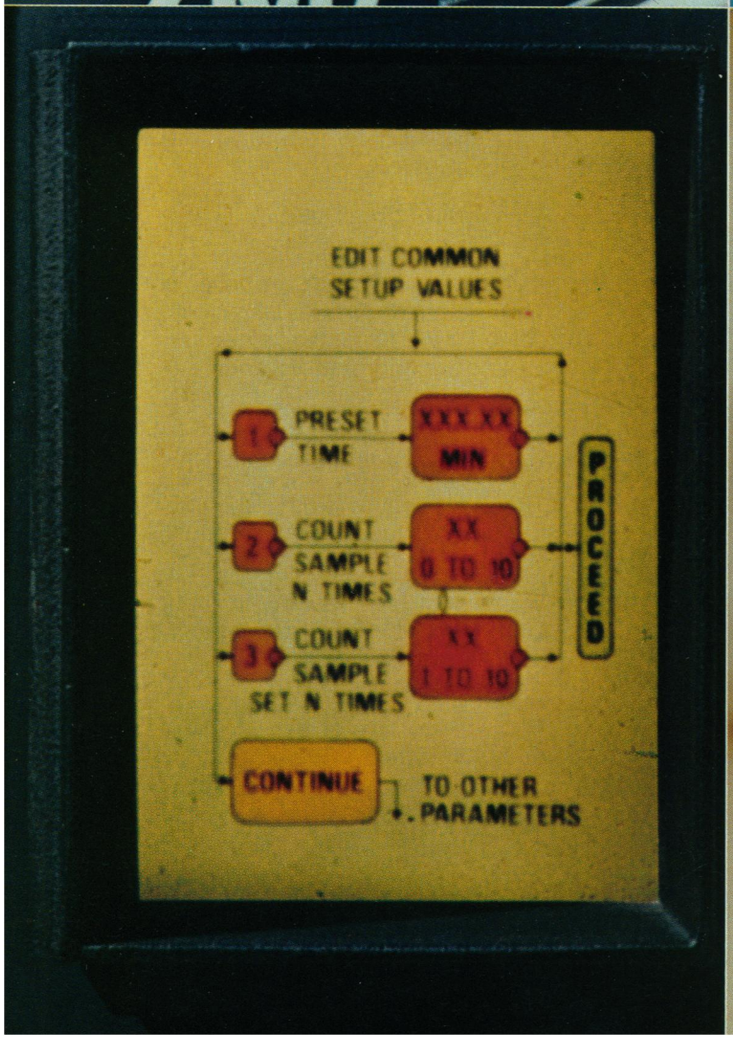
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LETTERS

Recombinant DNA: NIH Guidelines

Erwin Chargaff's and Francine R. Simring's letters (4 June, pp. 938 and 940) regarding recombinant DNA research require comment. Analysis of the history leading to, and the substance of, the guidelines for conducting this line of research (*1*) suggest that both of these critics overlooked important facts.

It is relevant to our comments that we were among those who first publicly expressed concern over the potential hazards of recombinant DNA experiments (*2*, *3*); we were members of the organizing committee of the Asilomar Conference (*4*); neither of us is a member of the National Institutes of Health (NIH) Program Advisory Committee on Recombinant DNA, although we have been active commentators on that committee's efforts to develop guidelines; and one of us is, and one is not, pursuing recombinant DNA experiments in our own laboratories.

Chargaff questions the propriety and legitimacy of NIH's role in formulating guidelines for recombinant DNA research. Certainly the principal biomedical research arm of the United States must be concerned with the health of laboratory workers and the public at large. Even if Congress or another governmental agency had intervened early and assumed responsibility in the area of recombinant DNA research, it is not conceivable that policy could properly be formulated without the involvement of NIH and informed members of the scientific community. Acceptance of responsibility in this matter by the past and present directors of NIH was courageous, farseeing, and proper; moreover, the directors and the consultants who labored diligently to produce the guidelines deserve our gratitude.

Contrary to the implications in the letters by Chargaff and Simring, the discussions leading to the guidelines were directed toward eliminating or minimizing real and imagined hazards, rather than balancing benefits and risks. The only certain benefit is increased knowledge of basic biologic processes; the predicted benefits for medicine, agriculture, and industry will follow only upon this increased knowledge. It was concern for the potential risks with recombinant DNA that led a group of scientists involved in this research to call for a voluntary deferral of certain experiments (*3*). The guidelines either proscribe such experiments or require extremely stringent containment measures for them. Indeed, the list of experiments in the proscribed category was extended between 1974 (*3*) and the Asilomar Conference report (*4*) and is even further enlarged in the guidelines (*1*).

Permissible experiments under the guidelines are classified according to the best available estimate of potential risk. Increasing potential risk requires increasingly stringent biological and physical containment measures. Not all recombinant DNA experiments yield "new" organisms; recombination between the DNA's of organisms known to exchange genetic information in nature do not add uniquely man-made species to the biosphere. In these cases, the guidelines follow the general principle that the experiments are to be carried out under previously defined conditions for handling the most hazardous parent of the recombinant. When DNA from species that are not known to exchange genetic material in nature are recombined, additional precautions are required. And it is precisely concerns of the kind raised by Chargaff (for example, the unpredictable consequences of intestinal colonization by organisms carrying potentially harmful genes) that led to such special precautions. Admittedly, the estimates of potential hazard are presently conjectural and controversial. But it is precisely for this reason that the requirements specified in the guidelines are more stringent than most scientists estimate are required for safety.

The adequacy of the containment prescribed for permissible experiments is then the central issue. Estimates of achievable containment levels must be based on available facts. We share with Chargaff the belief that it is unacceptable to harm others. But the recommended procedures are not "smokescreens." The P3 and P4 levels of physical containment are designed specifically as controls on accidental dispersal and human errors; they are defined in detail in the guidelines, and there is documented experience on which to judge the efficacy of these facilities.

With regard to biological containment, the encompassing description of *Escherichia coli* as the "predominant facultative species in the large bowel" in Chargaff's letter is misleading. The guidelines require the use of strain K12 of *E. coli* or disabled derivatives of it. Strain K12 is not a predominant species in the large bowel; indeed, the available evidence indicates that *E. coli* K12 rarely establishes itself as a viable resident in the human gut (*1*). The disabled derivatives of K12 must pass strict tests to establish that they are unable to live in natural environments. Only thereafter can they be certified by the NIH Advisory Com-

mittee for use in experiments requiring high levels of containment. The reports from the April 1976 meeting of the Advisory Committee suggest that the committee will be very cautious in its evaluation and certification of such systems. And, contrary to Chargaff's and Simring's statements, the problem of secondary natural recombination of foreign sequences, either out of the original host and into common enteric organisms, or between the experimental vectors and naturally occurring organisms and vectors, has been central to the discussions leading to definitions of disabled host-vector systems. In fact, the guidelines themselves describe and deal with those problems. They require that data regarding the chance of spread of the foreign DNA (by survival of a host cell or secondary recombination) in particular environments must be supplied; the probability of such spread must be less than 10^{-8} before certain experiments are permitted. Probabilities on the order of 10^{-8} afford a high level of confidence for achieving meaningful containment, considering the small numbers of organisms that could possibly escape as a result of human errors or flaws in physical containment. Thirty years of study of the genetic chemistry of *E. coli* K12 provides confidence that such levels of containment can be achieved. While it is important to investigate alternatives to *E. coli* K12, it is not at all certain that useful and safer organisms exist. Predictions about the existence of rare and fastidious organisms unable to exchange DNA with common organisms inhabiting man or other living things are highly speculative.

If Chargaff and Simring had examined the massive and readily available correspondence, minutes, and documents accumulated over the last 3 years, they would have recognized that precisely those matters they claim were disregarded were discussed in considerable detail. (This documentation has been collected by the Massachusetts Institute of Technology Program in Oral History of Sciences.) The charge that discussions have been "permeated by the assumption that the work will go ahead" or that we can "act now and learn later" is inconsistent with the 1974 moratorium and with the acceptance at Asilomar, and in the guidelines, of the principle that certain experiments should be deferred. The essence of the development of the guidelines by the NIH Advisory Committee was a discussion of alternative containment specifications, including those mentioned by Simring. Simring fails to point out that the letters to the director of NIH from eminent scientists

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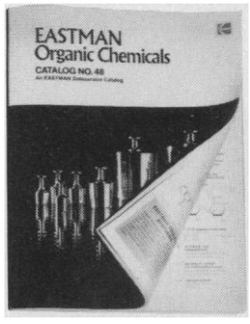

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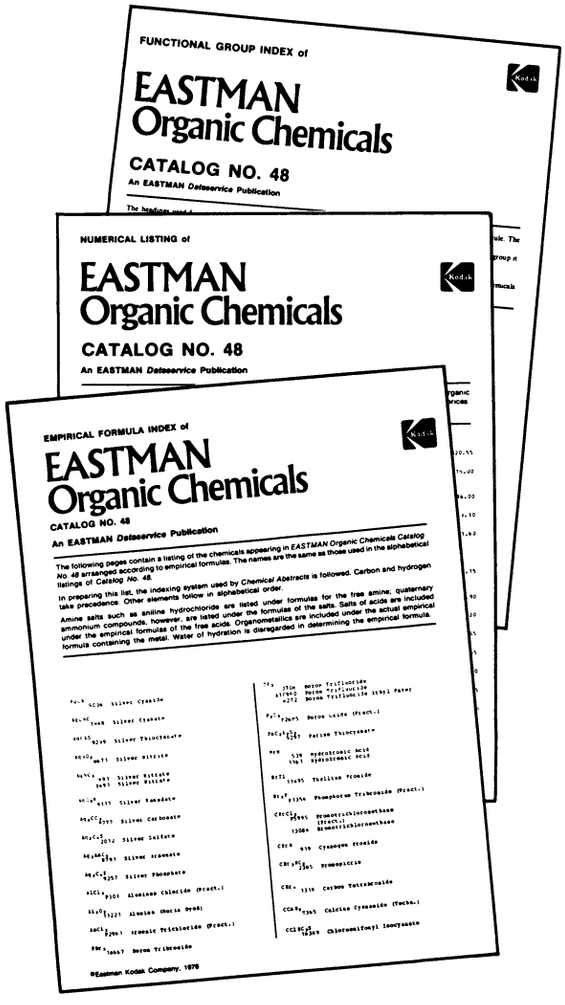

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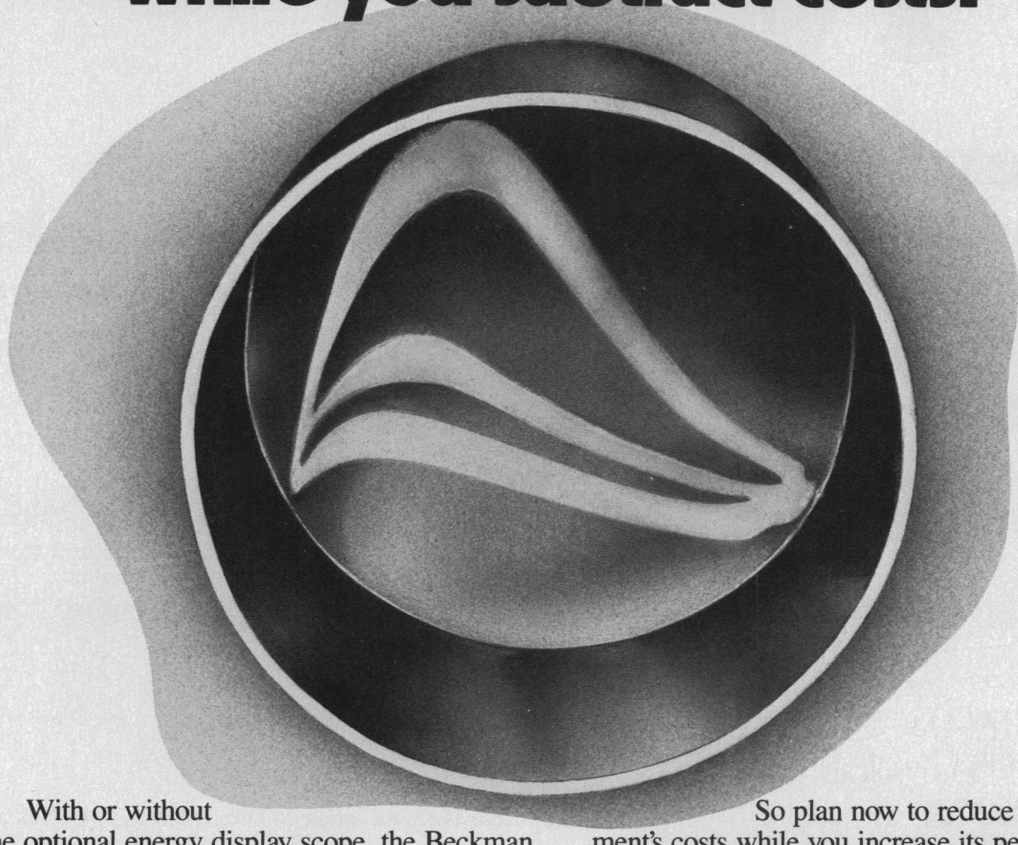

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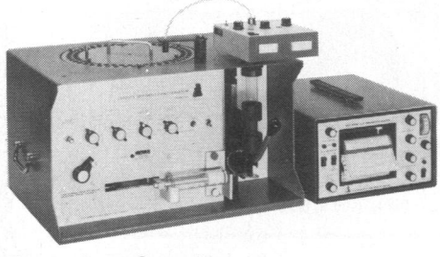
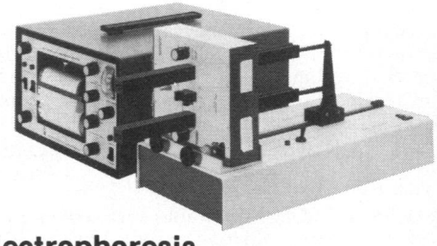


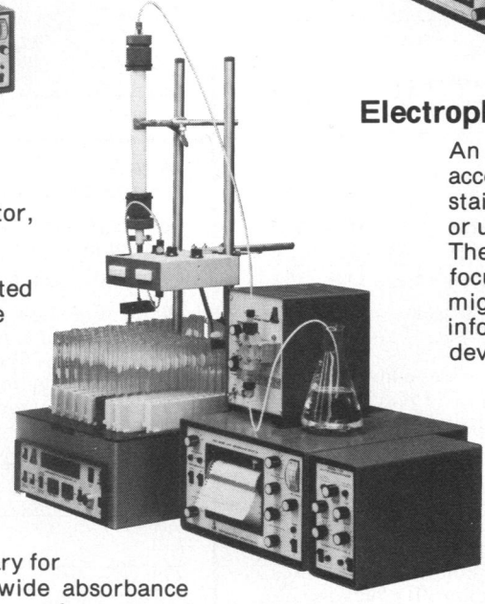
Density Gradients.

With an accessory gradient fractionator, the UA-5 automatically produces a continuous absorbance profile of the centrifuged gradient as it is fractionated into test tubes. There is no detectable loss of zone resolution.



Electrophoresis.

An inexpensive gel scanning accessory quantitatively scans stained gels at visible wavelengths or unstained gels at UV wavelengths. The UA-5 also scans ISCO isoelectric focusing columns repeatedly during migration to give you precise information about the separation as it develops.



Liquid Chromatography.

The UA-5 has the sensitivity, stability, and response speed necessary for high performance LC, along with the wide absorbance ranges and cold room reliability important for conventional chromatography. It operates at 13 wavelengths (two at a time), has a built-in 10 cm recorder, automatic scale expansion, and the exclusive ISCO Peak Separator which deposits different peaks into corresponding test tubes of an ISCO fraction collector.

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16 July 1976, Volume 193, Number 4249 **SCIENCE**

The Impact Statement—Part II

Since 1970, the Council on Environmental Quality has received environmental impact statements on nearly 7500 actions proposed by federal agencies. The CEQ's experience as overseer of the EIS process, under the National Environmental Policy Act, long ago confirmed several criticisms raised by D. W. Schindler earlier in this space ("The impact statement boondoggle," *Science*, 7 May): many EIS's are too long, they often include extraneous material that is neither analytical nor predictive, and the scientific quality of most is below desired standards.*

But to conclude, from these valid objections, that the impact statement process has "backfired" or that it has placed "the advancement of the scientific method . . . in jeopardy" ignores the real gains that have been made, both in protecting the environment and in elevating scientific knowledge to the decision-making level.

As a direct result of the EIS process, for example, the Trans-Alaska oil pipeline was redesigned to avoid adverse environmental impacts, plans for surface storage of nuclear wastes have been postponed for more thorough study, dams and other water resource projects that would have destroyed valuable natural systems have been modified or canceled, and literally scores of major highway and airport projects have been redesigned or eliminated. On this evidence alone, the EIS process has already proved its worth. Both benefits and problems are discussed in a recent CEQ report.†

Equally significant, as federal agencies adapt to EIS requirements, consideration of environmental impacts is becoming an integral part of decision-making rather than an afterthought. In addition, the EIS process opens up for effective review, both by the public and by government experts, decisions that were formerly made by individual agencies and their special-interest constituencies.

Despite these genuine gains, we are concerned with upgrading the scientific quality of impact statements. Accordingly, CEQ joined the Ecological Society of America and the American Institute of Biological Sciences in sponsoring a symposium on the biological evaluation of environmental impact at the 27th AIBS meeting in June.

The symposium specifically addressed the misconception that science as applied to the EIS process can be less stringent than science as applied to academic publication. The many distinguished participants agreed that the opposite is the case: evaluating environmental impacts and predicting environmental changes require a synthesis and understanding which exceed in difficulty the tasks that many environmental scientists have undertaken in the past.

Our relatively new policy on environmental protection expands opportunities for pure as well as applied ecological research. The $50 million in this year's budget for baseline studies on the Outer Continental Shelf is one example. The successful realization of that policy will also require scientists to address two principal challenges: first, to develop criteria for measuring the significance of environmental perturbations, and second, to describe these impacts in ways that are meaningful and useful to decision-makers and the public.

These problems do not, admittedly, fall into the neat disciplinary categories of traditional academic science. Yet our scientists have left the traditional pattern before. Their willingness to do so now in order to improve environmental impact analysis and government decisions will help discharge their responsibilities to society, as well as their responsibility for advancing science.—RUSSELL W. PETERSON, *Chairman, Council on Environmental Quality, Executive Office of the President, Washington, D.C. 20006*

*CEQ advised agency heads to remedy these problems in a memorandum of 10 February 1976.
†*Environmental Impact Statements: An Analysis of Six Years Experience by Seventy Federal Agencies*, Report of the Council on Environmental Quality, March 1976.

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there is second-rate science involved, this will be duly determined.

Though imperfect and evolving, the impact evaluation process has, on balance, been one of the more important advances in decades for the protection of environmental quality. It has been used at federal and state levels to bring the environment to the planning table and drawing board where before it was absent. It has opened the courts to environmental concerns. The issues that Schindler raises are largely issues for science itself, and not for the environmental impact evaluation process.

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1. Council on Environmental Quality, *Environmental Quality—The Sixth Annual Report of the Council on Environmental Quality* (Government Printing Office, Washington, D.C., 1975), pp. 626–651.

The correspondence resulting from my editorial has shown that most readers grasped the spirit of my comment—that is, to attempt to stimulate a widespread scrutiny of impact science. A few did not correctly judge my intentions.

Among these were "pure" ecologists, who continue to ignore current environmental problems in order to pursue their passion for determining the niobium content of horsefeathers, or whatever.

Another large group of correspondents were impact scientists, most of whom agreed with the editorial, but who almost universally said, "Our impact statements are not like that." Many (not all) of the examples they enclosed were.

I have no quarrel with the impact study concept and do not mind that my own work is regarded as such by most scientists [for example (*1*)]. But much of the work that I have seen has not been of the rigorously documented sort described by Auerbach *et al.*

Loftin's last sentence describes a general philosophy of science with which one must agree. Science has traditionally developed as he describes, and one is confident that correct results will always come eventually, leaving only a relatively harmless pile of worthless papers, wasted man-hours, and broken test tubes behind. But we cannot afford to let impact science follow tradition. The legacy will not be broken test tubes, but hopelessly and permanently crippled ecosystems.

It is this belief that leads me to think that impact work should be published, even if it is after the decisions relevant to a particular study have been made. We must develop an international, accessible, and comparative body of impact-related literature in order to allow extrapolation and generalization. It is simply not economical to treat each impact as though it were entirely unique. Synthesis will be impossible as long as relevant scientific work is hidden in inaccessible impact literature. The long-term loser will be the North American public, already subjected to high resource prices, which must be still higher if impact statements are not efficiently done.

Finally, I believe that it is time for educational institutions to pay more attention to the multidisciplinary training that good impact science demands. We would benefit in the long run if some impact dollars were diverted into such training programs instead of being applied to immediate problems.

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References

1. D. W. Schindler, *Science* **184**, 897 (1974).

The Origin of Pulmonate Land Snails

It has been brought to my attention that in my review (7 May, p. 547) of V. Fretter and J. Peake's *Pulmonates* (*1*) I ignored evidence that a higher limmic Basommatophora (including major freshwater families such as the Physidae, Lymnaeidae, Planorbidar, and Ancylidae) almost certainly are derived from air-breathing forms rather than the other way around (*2*). Thus the presence of air in the lung would not be a "preadaptation" but a holdover, and I am probably wrong in having criticized Ghiretti and Ghiretti-Magaldi. Fretter's statement that the terrestrial pulmonates originally came from the sea via fresh water is still probably valid, but what group or groups were involved is an enigma.

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References

1. V. Fretter and J. Peake, Eds., *Pulmonates*, vol. 1, *Functional Anatomy and Physiology* (Academic Press, New York, 1975).
2. W. D. R. Hunter, in K. M. Wilbur and C. M. Yonge, Eds., *Physiology of the Mollusca* (Academic Press, New York, 1964), vol. 1, pp. 1 and 93.

RESEARCH NEWS

(Continued from page 222)

Roth, however, argues that the effect is part of a more complex regulatory mechanism. He contends that the observed proteolytic activity of insulin is much too small to account for the observed decrease in receptors. He finds, furthermore, that the insulin must bind to the receptor before the effect is observed, and that anything which disturbs the normal functioning of the cell—such as a reduction in temperature, inhibition of energy production, or inhibition of protein synthesis—stops the loss of receptors. Roth thus argues that high concentrations of insulin somehow provoke an acceleration of loss of receptors. This feedback control prevents the cell from being overstimulated by the large amounts of hormone that are present. Similar regulatory mechanisms, he adds, have now been observed for other hormone-receptor systems. Many of Roth's and Cuatrecasas's observations are obviously in direct conflict, and the source of this conflict is not yet clear.

A Change in Equilibrium

But reducing the number of receptors does not provide precisely the same equilibrium, Roth argues, and glucose metabolism becomes slightly deranged. The greater the reduction in number of receptors, furthermore, the greater the degree of derangement. This possibility, he concludes, suggests the need for a reappraisal of the manner in which insulin is used therapeutically.

Perhaps one of the most important observations from the study of receptors is the recognition that the concentration of circulating insulin and the number of receptors can be controlled by diet. Roth, Phillip Gorden of NIAMDD, and Juanita A. Archer of Howard University made a study of insulin binding in 11 obese individuals. Their findings were consistent with other studies in that insulin concentrations were high and the number of receptors in monocytes was low. They also found, however, that restricting the caloric intake of the subjects produced a reduction in insulin concentrations and an increase in the number of receptors. The return to normal occurred with only a modest weight loss. Blecher and Goldstein have similarly observed normal binding of insulin and glucagon to monocytes from three obese diabetics who were following a rigid diet. And Olefsky and others have observed the same effects in rodents.

This finding could have major implications for maturity-onset diabetics—80 percent of whom are overweight. Roth's results, in particular, indicate that a major cause of insulin resistance—and thus of derangements in glucose metabolism—in these individuals is overeating. This suggests that much better control, and perhaps even complete control, of diabetes can be achieved by close regulation of the diet. This possibility is buttressed by the results of several clinicians, such as Jack H. Davidson of the Emory University School of Medicine, who have demonstrated that most maturity-onset diabetics can control the disease by regulation of their diet. This approach may receive even more attention in the future as a result of growing disenchantment with use of drugs and insulin for control of diabetes.

Oral Agents Increase Binding

Oral antidiabetic agents such as the sulfonylureas also have a surprising effect on insulin binding. Olefsky and Reaven have found that monocytes from untreated, nonobese, maturity-onset diabetics who exhibited fasting hyperglycemia had a reduced number of insulin receptors. When these subjects were treated with the sulfonylurea chloropropamide, Olefsky and Reaven observed, their fasting hyperglycemia was reduced and the number of insulin receptors increased, although neither returned to normal. Previous studies have shown that chloropropamide does not alter insulin secretion, and Olefsky and Reaven's evidence indicates that it does not interact directly with the receptors. It thus seems likely that the drug in some manner affects the control mechanisms that regulate the number of receptors.

There are, of course, probably other defects associated with maturity-onset diabetes and obesity. This is almost certainly the case with the large adipocytes, where most of the investigators agree that the principal defect lies in the intracellular metabolism of glucose. This may also be the case with some of the more severe forms of maturity-onset diabetes. But for the majority of the cases of maturity-onset diabetes, which involve mild symptoms in association with obesity, a majority of the investigators might now agree that one of the major causes is a defect in insulin binding to receptors on the cell surface.

—THOMAS H. MAUGH II

Erratum. An article about plant biochemistry in the Research News section of the 28 May issue of *Science* made reference to "the late K. Müller of Germany." Müller is alive and well, and *Science* regrets any problems that might have arisen from this mistake.