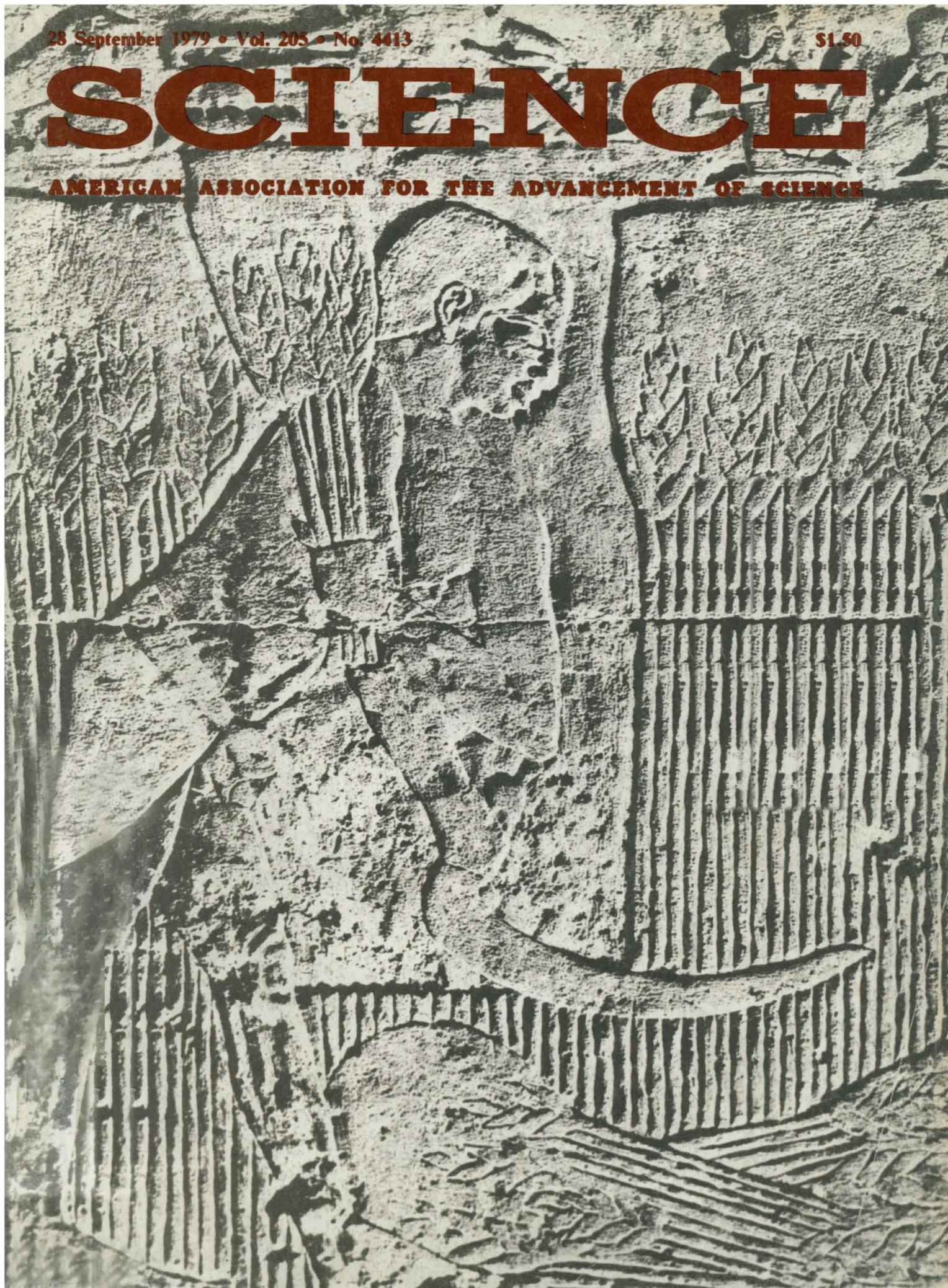


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COVER

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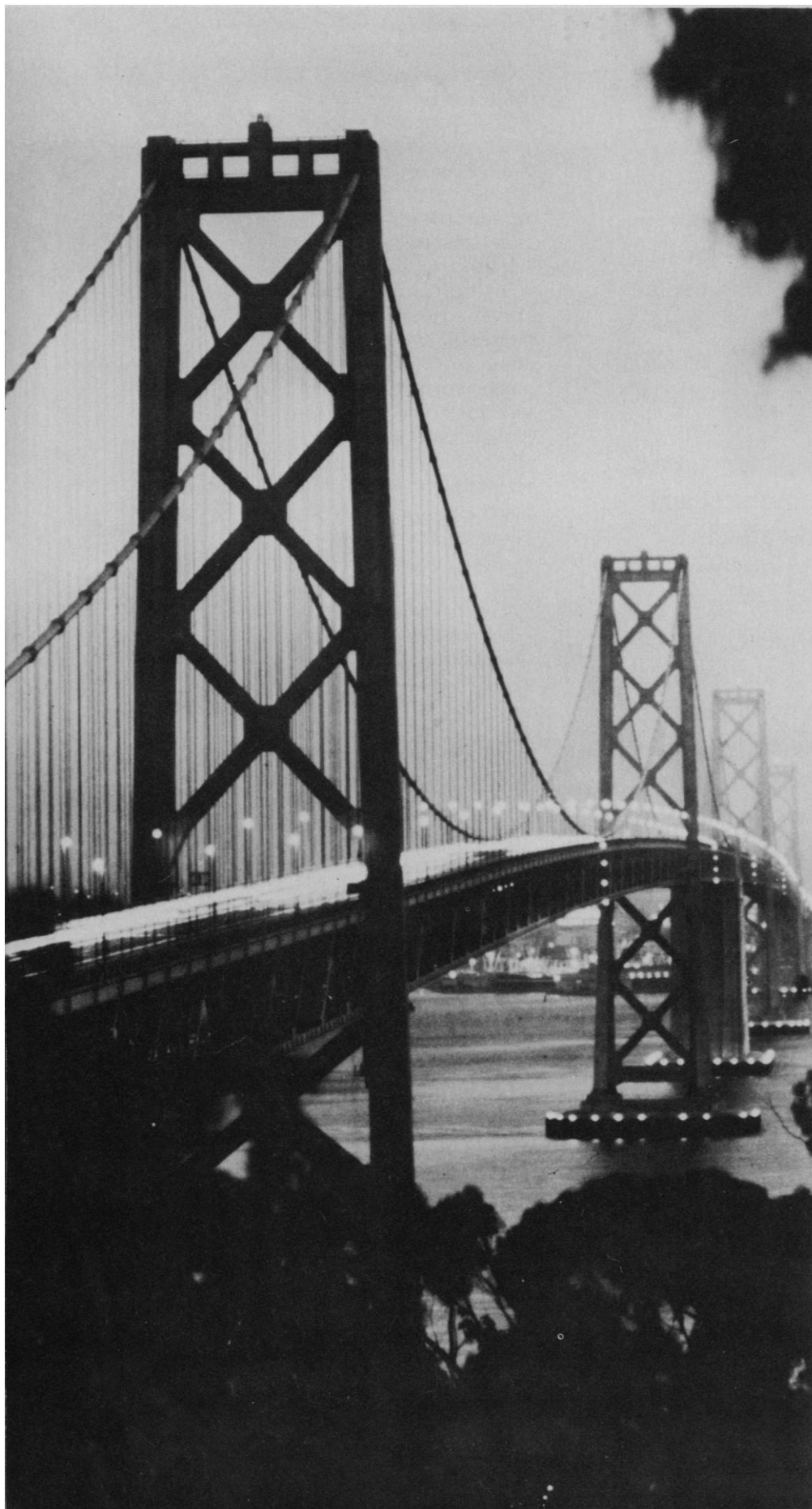
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Scientific findings of the unique, first-of-its kind Dispute Resolution Conference sponsored by the American Farm Bureau Federation run counter to the position of the Federal EPA which placed a temporary ban on most uses of the pesticide in March.

To those who still believe 2,4,5-T should be banned we commend the just released Conference report. Especially the scientific conclusion on the Alsea, Oregon epidemiology study on which the EPA based its action to ban. The scientists agreed that the report has such deficiencies that no conclusions are possible with regard to alleged abortion-causing effects of 2,4,5-T.

We compliment the scientists who took the necessary time to resolve issues now restricting the use of this important agricultural tool. The participants came

from the fields of medicine, toxicology, chemistry, ecology, agriculture. They represented the scientific concern of Canada, Italy, Sweden, New Zealand, Switzerland, France, Germany and the U.S.A.

But we wonder why many of the ever-vocal minority who seek a final end to the use of 2,4,5-T and other pesticides *declined to attend* or to join in the scientific dispute ... certainly an ideal forum for arguing their case with the world's scientific experts.

The full Dispute Resolution Conference report has been mailed to Members of Congress and other government officials ...we urgently direct their attention to this decisive, truly scientific consensus ...that 2,4,5-T is safe when properly applied.

It's important to understand that there is still no *scientific* reason to prevent the pesticide's use by literally hundreds of thousands of Americans who have depended on it for 30 years...in growing their crops, cattle, timber. For more information on the Conference findings, write American Farm Bureau Federation, 225 Touhy Ave., Park Ridge, IL 60068.



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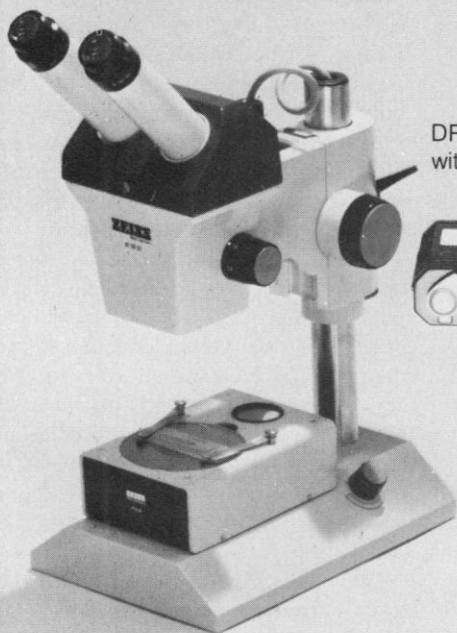
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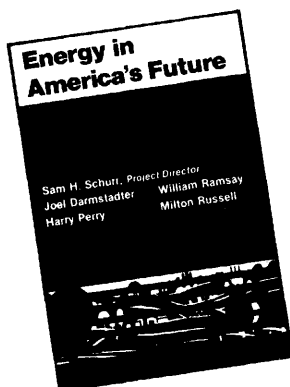
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## **LETTERS**

### **Dioxin Studies**

In reference to the article, "Agent Orange furor continues to build" by Constance Holden (News and Comment, 24 Aug., p. 770), I note that no mention is made of long-term studies of the effects of exposure to dioxin on human health that are being conducted by the National Institute for Occupational Safety and Health (NIOSH).

NIOSH, with cooperation from the chemical industry, major unions, and the Department of Defense, is compiling a registry of the population of chemical workers in the United States who have had documented exposure to the constituents of Agent Orange, such as 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), either in the manufacture of 2,4,5-trichlorophenol (2,4,5-T) and other herbicides or in industrial accidents. Once this registry has been developed, NIOSH plans to evaluate trends in mortality of the exposed workers and, if the data permit, will consider conducting morbidity and reproductive studies. Because the manufacture of TCDD-contaminated herbicides began in this country as long ago as the mid-1940's, this registry of several thousand exposed workers should provide information on the effects of dioxin exposure that will be relevant to the present and future concerns of Vietnam veterans.

ANTHONY ROBBINS

*Office of the Director,  
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### **Nitrate and Nitrite:**

#### **Origin in Humans**

The report by Witter *et al.* (27 Apr., p. 411) on the use of labeled nitrate ( $^{15}\text{NO}_3^-$ ) to investigate nitrate pharmacokinetics in humans and the rat represents an important contribution to the literature, and directly substantiates several facts that were previously known only through indirect measurements. The most relevant of these is that nitrate is absorbed primarily in the upper portion of the small intestine, and that small quantities can reach the lower intestinal tract via the intestinal tube or by reverse diffusion from blood. The significance of these new findings to the interpretation of our earlier report (30 June 1978, p. 1487) on excess nitrate synthesis in humans, and to our hypothesis of intestinal


heterotrophic nitrification requires further comment. We believe these new findings support our hypothesis and demonstrate that reverse diffusion could not account for the concentrations of nitrate and nitrite in urinary, ileal, and fecal fluids.

The elimination of nitrate from the body via excretion in urine has been followed in our laboratory in young and old individuals on a variety of diets over periods of 1 month or longer. We are currently working on a study in which 24-hour urine samples have been collected for a consecutive period of 80 days. The most characteristic feature of urinary excretion of nitrate by individuals on our formula diets is the extreme variability from day to day. The daily nitrate intake, as we reported, ranges from approximately 75 to 150 micromoles, while the output exceeds the input by factors of 2 to 60. Over a period of 80 days, the average excess nitrate excretion of individuals on a soy diet is greater than 5 grams. Verification of our observations has recently appeared (1) and has also been communicated in correspondence (2).

Our studies, and those of others who have conducted careful experiments on nitrate metabolism, indicate the following highly simplified picture: nitrate clearance from blood after an oral dose involves a distribution phase of 2 to 5 hours to peak concentration in urine and saliva, and a clearance phase with a half-life of approximately 8 hours (3). It is also well known that nitrate rapidly equilibrates with extracellular water (4). Therefore, if one assumes no further entry of nitrate, the body could clear its pool in approximately 48 hours, independent of the initial concentration of the pool, since clearance is first-order in concentration of nitrate. This is, in fact, the observed result of the studies previously cited and is also verified by our unpublished observations of blood nitrate concentrations in fasting individuals.

While the  $^{15}\text{N}$  technique would appear to be extremely valuable for short-term distribution studies, the short half-life of  $^{15}\text{N}$  limits its experimental use to time periods that are shorter than the distribution phase of nitrate given orally in vegetables or vegetable juices. Another deficiency is the lack of resolution of the method, which did not permit the experimenters to distinguish, for example, intestinal contents from intestinal wall. A third deficiency, as noted by the authors, is the lack of ability to distinguish between nitrate and its reaction products. This is a serious difficulty in the inter-





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pretation of the study by Witter *et al.*, since they did not attempt to measure the specific activity of  $^{13}\text{NO}_3^-$  in the various compartments. It is quite probable that some of the oral dose is converted to  $\text{NO}_2^-$  and the ammonium ion ( $\text{NH}_4^+$ ) or is simply taken up by bacteria along the mouth-esophagus-stomach-small intestine route, or both. The dose of  $^{13}\text{NO}_3^-$  given to the rats is equivalent to approximately  $0.33 \times 10^{11}$  molecules. The largest amount of the dose found in the ileum, 4 percent, would be on the order of one molecule of nitrate per intestinal bacterial cell! Therefore, some caution should be exercised in the interpretation of these studies, and the application to nitrate pharmacokinetics should be interpreted on the basis of additional chemical studies.

Witter *et al.* suggest in their abstract that their results "may be a better explanation of urinary, ileal, and fecal concentrations of nitrate and nitrite . . . than a bacterial nitrification in the intestines." I fail to see how they can arrive at this conclusion on the basis of studies that measure only nitrate flux. Since earlier pharmacokinetic studies (4) demonstrated that nitrate is distributed in extracellular water, this would account for the  $^{13}\text{N}$  label in the carcass. The authors own studies on humans and the rat demonstrate that there is no mysterious concentrated nitrate depot, and therefore, any "stored" nitrate would be cleared at a rate determined by the plasma concentration. Nitrate in urine in an amount greater than that of oral intake cannot possibly be accounted for by movement of nitrate into the intestine and concomitant formation of nitrite. This would result in destruction of nitrate, not de novo synthesis.

Witter *et al.* argue that the ileal and fecal nitrite and fecal nitrate may be due to reverse diffusion. This may, of course, be a partial explanation of our findings, and could have been the logical conclusion if nitrate had also been found in the ileal fluid. It was, however, the absence of nitrate in this fluid that led us to propose the mechanism of heterotrophic nitrification since, in years of investigation on bacterial reduction of nitrate, we have noted only partial conversion to nitrite. As far as I am aware, neither we nor others have seen a sample of saliva containing nitrite but not nitrate.

Finally, although our original proposal of intestinal nitrification was made on the basis of indirect evidence, we have now isolated a variety of microorganisms from ileostomy patients and from intact human ilea which are capable of forming nitrite from  $\text{NH}_4^+$  or amino acids under



conditions similar to those of the intact ileum or caecum (5). We have also presented results (6) demonstrating a dietary effect of different protein sources on excess nitrate synthesis.

It thus appears that exposure to nitrite is unavoidable, and we should seek to block the synthesis of *N*-nitroso compounds from nitrite through intake of such agents as ascorbic acid and  $\alpha$ -tocopherol.

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Tannenbaum proposes, on the basis of input-output studies in volunteers, that bacterial nitrification can occur in the human intestine and produce at least 5 grams of  $\text{NO}_3^-$  per month. A number of points should be clarified before this concept of heterotrophic nitrification by intestinal bacteria, to the extent indicated by Tannenbaum, can be accepted. These points are as follows:

1) Other  $\text{NO}_3^-$  and nitrite ( $\text{NO}_2^-$ ) balance studies involving rats (1), dogs (2, 3), goats and rabbits (4, 5), humans (3), and the reference for dogs (6) cited in Tannenbaum's letter have shown incomplete recoveries of  $\text{NO}_3^-$  (and  $\text{NO}_2^-$ ) in the urine after ingestion or injection of these ions but never an excess of  $\text{NO}_3^-$  or  $\text{NO}_2^-$ . Also, others (7) have estimated that less than half of the ingested  $\text{NO}_3^-$  is recovered in human urine.

2) Other workers have failed to detect  $\text{NO}_3^-$  or  $\text{NO}_2^-$  in human ileostomy (8) or fecal (3, 9) samples.

3) It has been noted in several reports (10, 11) that quantitation of  $\text{NO}_3^-$  and  $\text{NO}_2^-$  in various diets is difficult. Therefore, it is difficult to rule out the possibility that ingested  $\text{NO}_3^-$  and  $\text{NO}_2^-$  "hidden" from reaction in the Griess test may, nonetheless, be made available or released during digestion. In fact, analytical considerations have prevented others (7) from speculating on the endogenous formation of  $\text{NO}_3^-$  in humans.

4) Nitrification (to the best of our

knowledge) has a requirement for oxygen ( $\text{O}_2$ ) (12), but the gut is generally considered to become progressively more anaerobic from the duodenum to the anus (13). Bacteria may have access to small amounts of  $\text{O}_2$ , presumably from swallowed air (13), and the possibility exists (at least in ruminants) that microorganisms attached to the gut epithelium may derive  $\text{O}_2$  from the blood supplied to the intestines (13). However, this latter point (to our knowledge) has never been proved. Tannenbaum indicates in his letter that nitrification can occur in vitro under conditions that are "similar to those of the intact ileum or caecum." We have looked at the manuscript of Gomez *et al.* (14) (personal communication from Tannenbaum) and fail to find how the in vitro growth conditions they describe are similar to the intact ileum or caecum. We also feel that "atmospheric" (in addition to the microbial, physiological, nutritional, and biochemical) conditions of the intestinal tract are not like those of a "sewer" as proposed by Tannenbaum *et al.* (Reports, 30 June 1978, p. 1487).

5) It appears unlikely to us that oxidation of ammonia (or other nitrogen compounds) to  $\text{NO}_3^-$  could occur in the gut at the rate of 5 grams per month. For example, if one assumes that (i) 50 percent of intestinal  $\text{NO}_3^-$  formed via heterotrophic nitrification is absorbed and eventually excreted in the urine; (ii)  $\text{NO}_2^-$  is the precursor of  $\text{NO}_3^-$ ; (iii) *Pseudomonas aeruginosa*, which may be found in the human intestinal tract, can form nitrite at an optimum rate of 2 milligrams of nitrite per day per gram of cells (dry weight) (12); and (iv) a typical bacterial cell (wet weight) averages  $4.7 \times 10^{-13}$  gram (16), then, to form 5 grams of  $\text{NO}_3^-$  per month would require about 200 grams (wet weight) of bacteria for heterotrophic nitrification. This seems improbable, since it would be equivalent to approximately  $4 \times 10^{14}$  bacteria and, as pointed out by Tannenbaum in his letter, there are  $0.33 \times 10^{11}$  bacteria in the rat. Estimates in humans range up to approximately  $10^{14}$  bacteria in the gastrointestinal tract (17). The vast majority of these bacteria, which are strict anaerobes or facultatively anaerobic bacteria, are found in the distal ileum and colon. The population of bacteria is very sparse in the upper intestinal tract, where Tannenbaum *et al.* (30 June 1978, p. 1487) indicate heterotrophic nitrification occurs. The oral cavity might be a site where heterotrophic nitrification could occur. However, as Tannenbaum *et al.* (18) has shown, bacterial nitrate reductase activity predominates in the oral cavity.

6) Other sources of urinary  $\text{NO}_3^-$ ,

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such as environmental oxides of nitrogen, should also be carefully considered (19). For example, if 6 liters of air are inhaled per minute and the air contains  $\text{NO}_2^-$  at a concentration of 1 part per million (ppm), approximately 0.5 gram of  $\text{NO}_3^-$  could be formed per month.

The observations in our *Science* report on the pharmacokinetics of labeled nitrogen ( $^{13}\text{NO}_3^-$ ) in humans and rats represent only a portion of the work that has been conducted using this isotope. Comment on our other work is also necessary, even though it has been accepted for publication in another journal (20). We believe our findings neither support nor absolutely negate heterotrophic nitrification by intestinal bacteria. We do believe they demonstrate that labeled nitrogen ( $^{13}\text{N}$ ) of nitrate and nitrite reaches the lower intestinal tract by ingestion with food and water and by passage from the bloodstream into the lower intestinal contents. Tannenbaum has selected only one mechanism, "reverse diffusion," from our report as a potential explanation for his ileal or fecal  $\text{NO}_3^-$  and  $\text{NO}_2^-$  results. Our experiments show that up to 24 percent of gavaged  $^{13}\text{N}$  from  $^{13}\text{NO}_3^-$  can reach the lower intestinal tract of conventional-flora (CV) rats within 1 hour. Passage down the intestinal tract combined with reverse diffusion and the as-yet-unconfirmed possibility of  $^{13}\text{N}$  reentering the intestine via the pancreatic or biliary secretions or both, and not simply "reverse diffusion" alone, were the basis for the last sentence in our abstract to account for ileal and fecal  $\text{NO}_3^-$  and  $\text{NO}_2^-$  values. We feel these mechanisms may better explain the results of Tannenbaum *et al.* (30 June 1978, p. 1487) because the fecal and ileal samples he reported were apparently from patients ingesting a "free choice, Western-style diet" and were not from the same group of volunteers who were on a restricted (low  $\text{NO}_3^-$ , or protein) diet. Consequently,  $\text{NO}_2^-$  in the ileal and fecal samples could have been of dietary origin.

Greene and Hiatt (6), cited by Tannenbaum, do suggest that  $\text{NO}_3^-$  in the blood equilibrates with extracellular fluid  $\text{NO}_3^-$  in dogs, and this may be responsible for our rat carcass  $^{13}\text{N}$  values. It may also be responsible for the fluctuation in urinary  $\text{NO}_3^-$  content noted by Tannenbaum *et al.* (30 June 1978, p. 1487), if the body's ability to retain  $\text{NO}_3^-$  is variable. We cannot agree with Tannenbaum's statement that "clearance is first-order in concentration of nitrate." Careful examination of the studies cited by Tannenbaum in his letter and of the above study (6) show that, although the published data show approximately first-order kinetics over



very restricted ranges of concentration and time, they are far from conclusive on this point. Indeed, since recycling of nitrate occurs through saliva and through the gut and since nitrate is metabolized by the bacterial flora, it is hard to see why clearance should be first-order, especially for near-normal endogenous levels.

The title of our *Science* report implies that the chemical form of  $^{13}\text{N}$ , once ingested, is not known. Although we are currently attempting to separate and characterize these  $^{13}\text{N}$  derivatives, our work with germfree (GF) rats may answer, to some extent, several of these questions raised by Tannenbaum, if one correlates our  $^{13}\text{N}$  results (after  $^{13}\text{NO}_3^-$  and  $^{13}\text{NO}_2^-$  are administered to GF and CV rats) with the chemical data when these same (unlabeled) compounds were given to GF and CV rats. Basically, GF rats do not appear to convert  $\text{NO}_3^-$  to  $\text{NO}_2^-$ . However, GF rats do chemically alter  $\text{NO}_2^-$  to excrete the  $^{13}\text{N}$  from gavaged  $^{13}\text{NO}_3^-$  more rapidly than do CV rats, and there appears to be more  $^{13}\text{N}$  in the intestinal tracts of CV rats than in GF rats. This suggests to us that the flora of conventional rats alters and metabolizes the  $^{13}\text{NO}_3^-$ . Also,  $\text{NO}_3^-$  and  $\text{NO}_2^-$  were never chemically detectable in the caeca of CV rats given 1000 ppm of sodium nitrate or 1000 ppm of sodium nitrite, whereas these ions were detectable in the caeca of GF rats fed the ions. We interpret these results to indicate that the nitrogen of ingested  $\text{NO}_3^-$  or  $\text{NO}_2^-$  reaches the lower intestinal tract in CV rats, but that these ions are chemically altered in the process. This bacterial reduction of available  $\text{NO}_3^-$  in the ileum may be responsible for ileal  $\text{NO}_2^-$  values as noted by Tannenbaum *et al.* (30 June 1978, p. 1487), rather than an oxidation of more reduced forms of nitrogen.

Our  $^{13}\text{N}$  data on GF and CV rats also show that, after intravenous injections of  $^{13}\text{NO}_3^-$  or  $^{13}\text{NO}_2^-$ , the  $^{13}\text{N}$  is present in both intestinal tissue and contents. In fact, most of the  $^{13}\text{N}$  (intravenously injected) present in the lower intestine of CV rats with ileocecal ligation (see table 2 in our *Science* report) was primarily located in the intestinal contents.

Although the idea of nitrification by intestinal bacteria is an extremely exciting concept, both biologically and in terms of the etiology of several types of human cancer, we feel the analytical, microbiological, and pharmacokinetic data to date are insufficient for such an assumption. This is essentially what prompted us to submit our report to *Science*. Our exposure to nitrite may be unavoidable, not because of bacterial heterotrophic nitrification, but because of our large in-

take of nitrate, which is known to be reduced to nitrite by alimentary tract bacteria. Whether the bacteria metabolizes nitrite to harmful or innocuous compounds remains to be determined.

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**Erratum:** In a letter to the editor (3 Aug., p. 447), Yvonne Brackbill writes (p. 448, column 2, line 11), "In the state of New York, two recent Court of Appeals decisions (27) found physicians negligent in failing to advise, or advise accurately, the pregnant women who consulted them to obtain such information." Reference 27 is to "Becker vs. Schwartz, 46 N.Y. 2d Ser., 401 (1979); Park vs. Chessin, *ibid.*" This statement is not correct. The Court of Appeals did not, in these cases, rule on negligence or lack thereof on the part of the physicians. The decisions were that, under certain circumstances, parents had the right to bring an action to determine whether they had received pertinent information. The court in no way discussed the validity of the particular claims in either case.

The Park vs. Chessin case was tried after the Court of Appeals decision, and the defendant physicians, including Chessin, were found not negligent. The Becker vs. Schwartz case is still awaiting trial.

**Erratum:** In the article "Dynamics of skeletal pattern formation in developing chick limb" by S. A. Newman and H. L. Frisch (17 Aug., p. 662), a clause was omitted. The clause should be inserted on page 667, third column, line 31, as follows: "[. . . respectively.] at  $t = t_0$ , but subsequently we would like the gradient in the  $z$  direction to be maintained, and thus require

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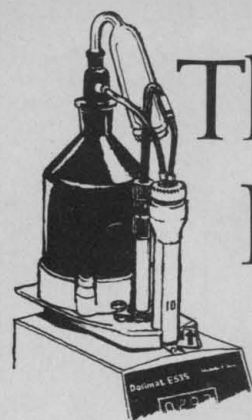


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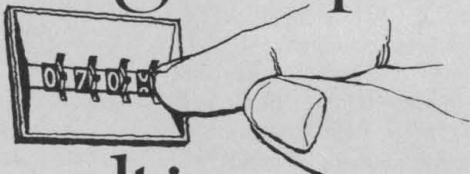
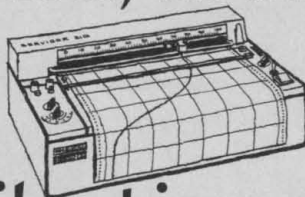
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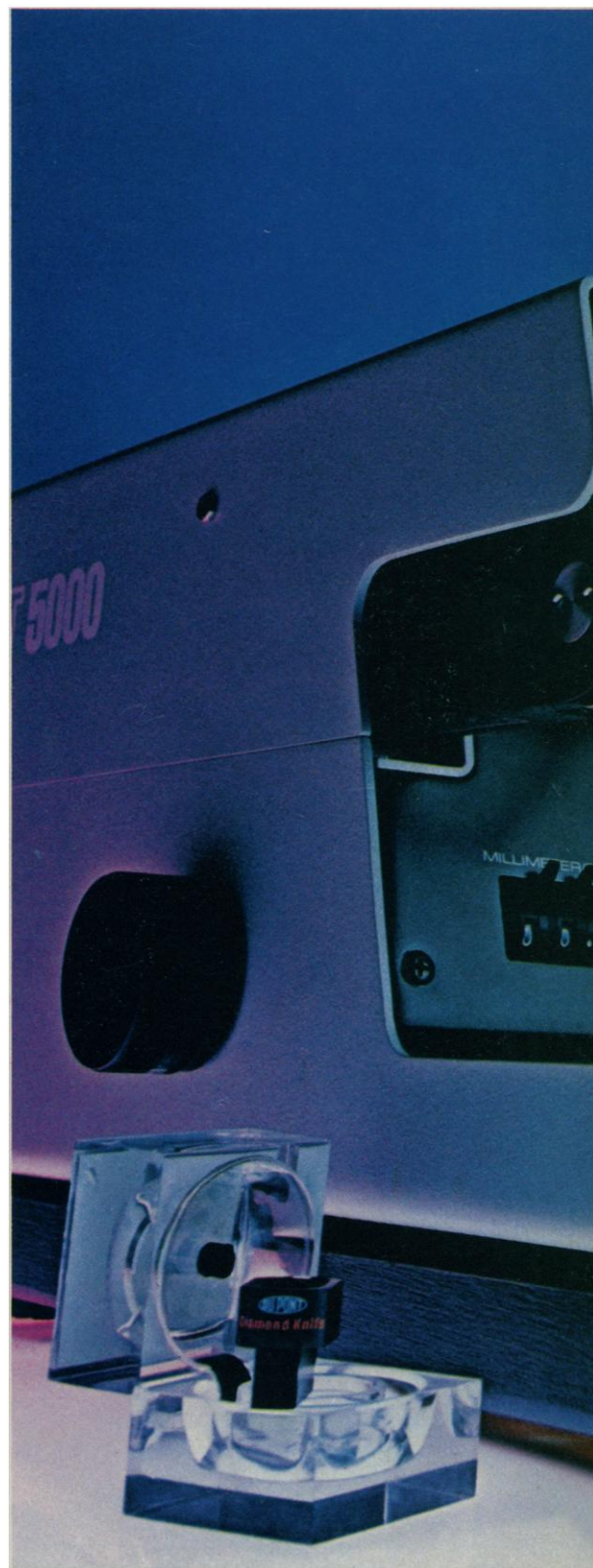
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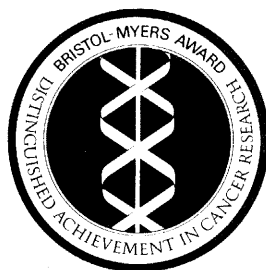
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## Science and the Politics of Development

Those who kept an eye on the recent United Nations Conference on Science and Technology for Development had a glimpse of what it means to mix science and technology with contemporary diplomacy. There can be no further doubt that science and technology are high cards in the new international political economy. If the Vienna meeting accomplished little else, it dramatized the North-South gap by pounding home the fact that three-fourths of the world's people account for only 3 percent of the world's research and development.

Although the Vienna meeting with its thousands of participants did not display the exquisite diplomatic theatre that scientists thought they might find in the city of Metternich, it mirrored powerfully the rising turmoil in the relationships between the struggling human majority and the preoccupied minority. The message from the developing nations was explicit: that they will no longer accept the trickle-down method of scientific and technological transfer that suits the advanced countries, and that access to both theoretical and practical knowledge looms large in their formulation of the new international economic order.

While the developing nations were cheerfully thrashing the advanced societies for allegedly hogging scientific and technological capacity and conspiring to create the brain drain, they also bracketed the superpowers—capitalist and socialist alike—as equally to blame for diverting scientific and technical expertise into a mindless arms race. It was an indictment with telling effect. As almost nothing else could, it etched the contradictions in the main trends of current history: surging self-consciousness on the part of the emerging majority, contrasted with a rush to the edge of night by the superpowers. The limits of knowledge in managing peaceful change have seldom seemed so clear.

The fruits of the Vienna conference, by most standards, were indeed modest: A sort of promise of future increased funding for science and technology for development, but hardly a guarantee; a new intergovernmental committee to add to the existing regiments of the United Nations, but with a stronger voice for the Third World; new plans for international information networks of science and technology; and a promised search for an assured system for financing the transition of the developing countries to substantial self-reliance in science and technology. If it doesn't sound like much it is because it was not much, relative to the scale and intransigence of the predicament. If the agenda had been arranged so that it dealt with matching advanced and appropriate science and technology to the priorities of the developing countries for human needs and industrial growth, instead of featuring intractable political squabbles and parading old grudges, the time would have been better spent.

The rhetoric of the United Nations is better suited to amplifying differences than to reaching accommodations. Quieter and better things will be done, now that the noise has abated, through bilateral projects, specialized agencies, industrial approaches based on partnership principles, and the work of concerned scientists of both North and South. For all the posturing at UNCSTD, it was not a total loss. Those who were there will not find it easy to dismiss the global dissatisfaction of which Vienna was an awkward but very human symbol. While it lasted, serious men and women on each side of the development divide were searching each other out and communicating.

In the near term, despite all that was said at Vienna, there will be no dismantling of the vast advantage in science and technology enjoyed by the advanced nations over the developing world. Modest steps will be taken and some good will be achieved on that scale. But these are times when the advanced economies are themselves troubled and preoccupied with their internal problems of inflation, energy, productivity, and the worries of an unstable peace. Meanwhile, a wind is rising.—WILLIAM D. CAREY



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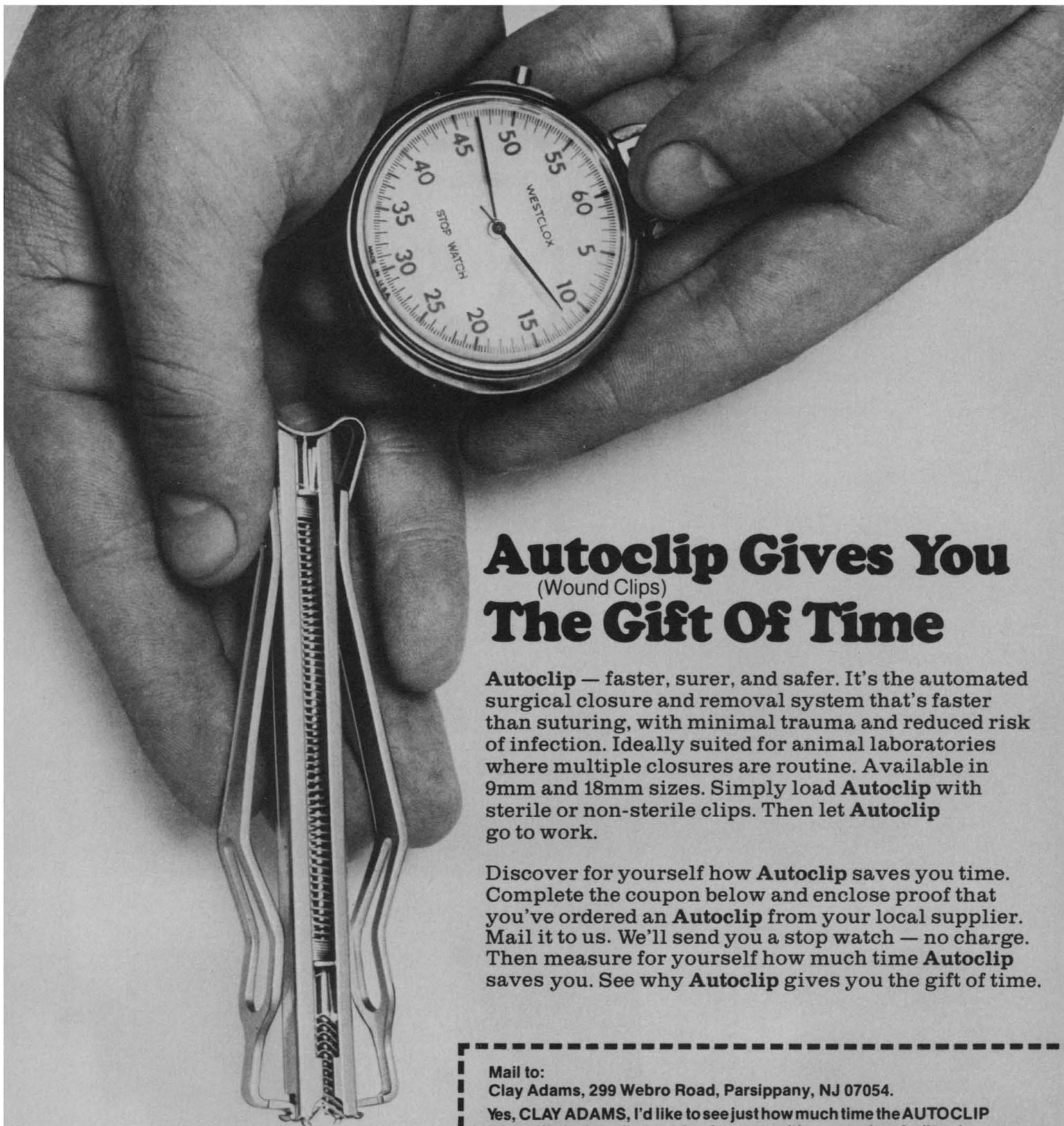
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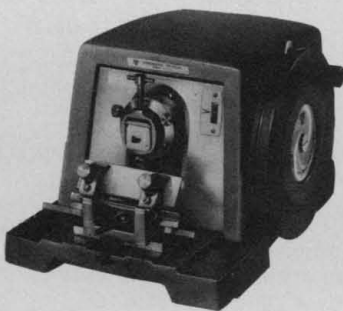
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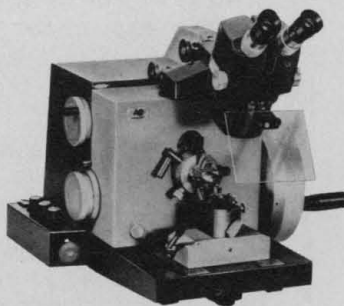


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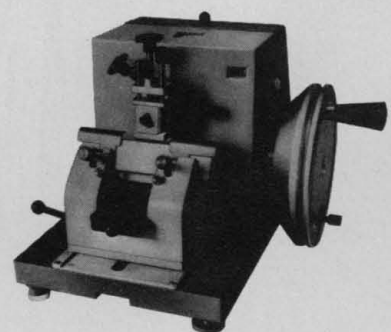
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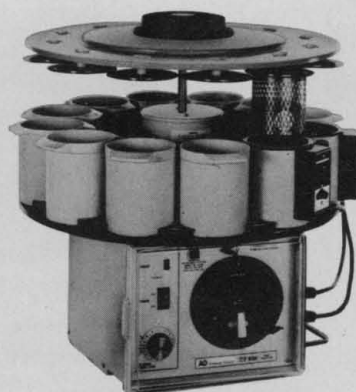
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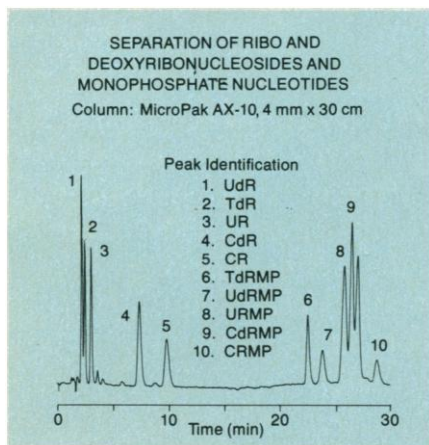




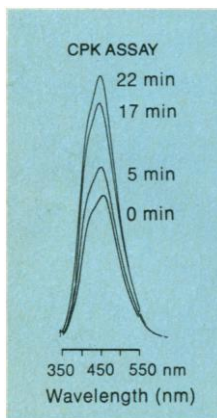
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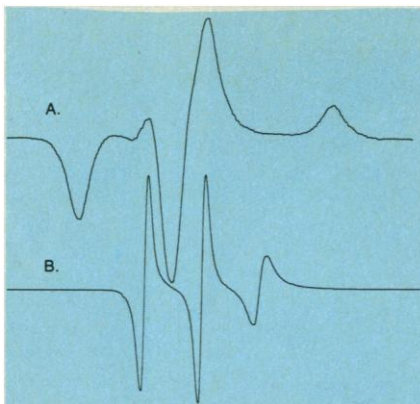
Ex = 340 nm, slit = 10 nm  
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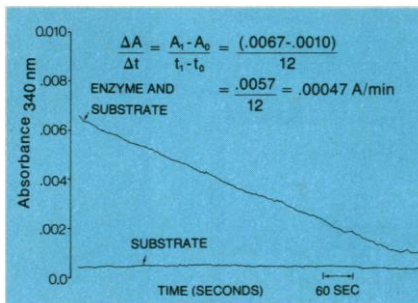
By using spin-labeled analogues or by identifying indigenous free radicals associated with cells, tissues, proteins, etc., the E-109E provides important structural and environmental information—often not obtainable by other methods. This information can be



Before (A) and after (B) administration of anesthetic.

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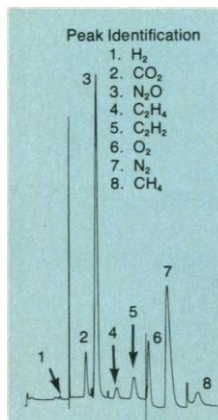
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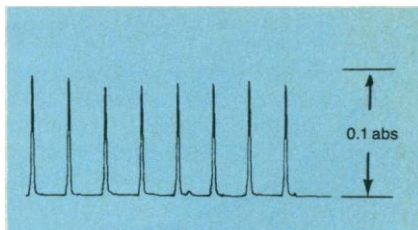
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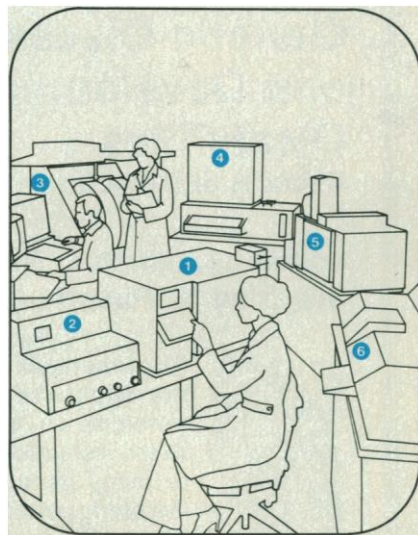
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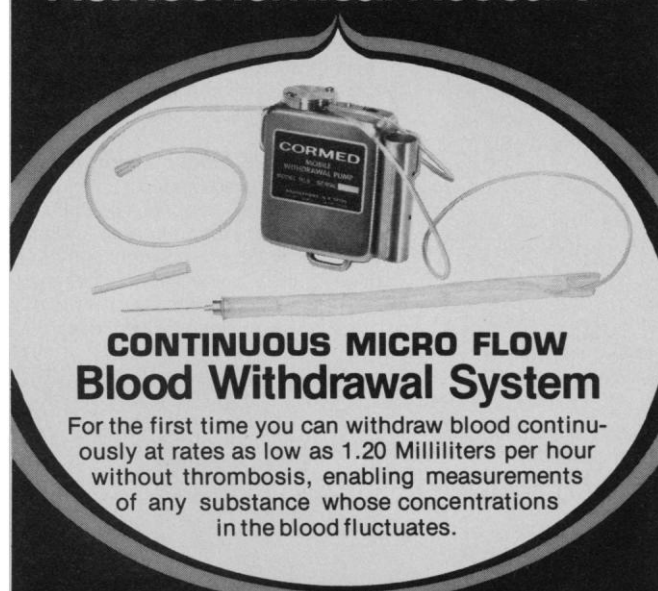
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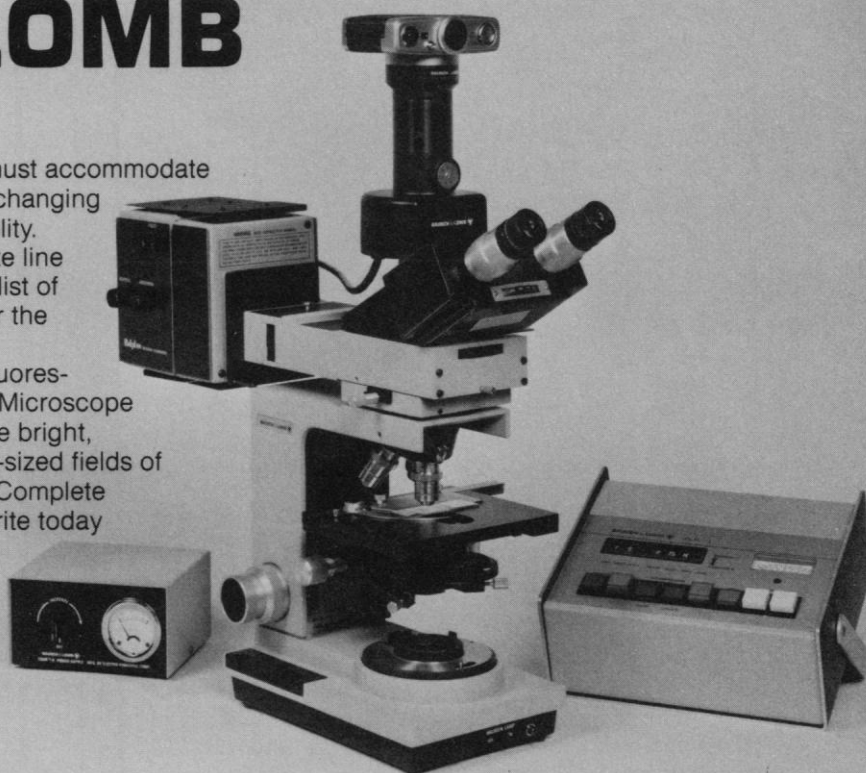
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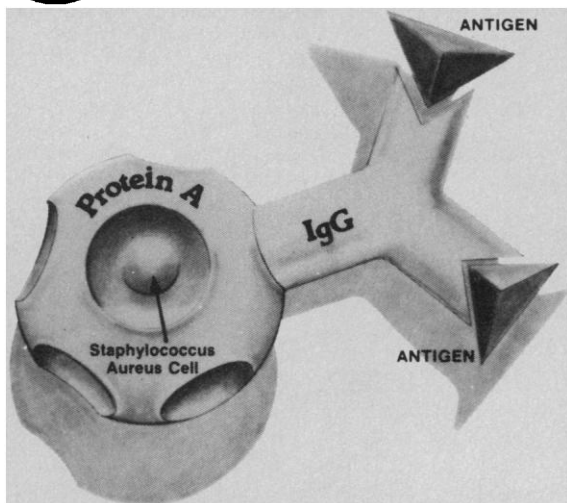
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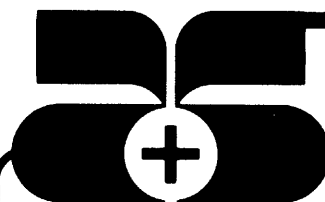
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