BIO-RESPONSE'S RESPONSE

To the editor:

The "Chronicle" section of March's *Bio/Technology* reported that Bio-Response was one of two contract producers to strike a deal on tissue plasminogen activator (t-PA). If taken at face value, this is indeed true. But the intent of our agreement with Retroperfusion Systems—in contrast to Damon Biotech's agreement with Yamanouchi Pharmaceutical—is not for the commercialization of t-PA specifically. Rather, the agreement is for the *investigational* use of our human t-PA in Retroperfusion's catheter technology.

This joint development program will form part of Bio-Response's pre-clinical and clinical testing program. Such tests will also be done in support of and in conjunction with human clinical trials that will commence following anticipated approval of our Investigational New Drug application, which soon will be filed with the Food and Drug Administration.

Alfred E. Daniel
President, Bio-Response Inc.
1978 West Winton Avenue
Hayward, CA 94545

LIGNINASE CREDIT (CONTINUED)

To the editor:

With interest I have read the comments by Ronald Crawford regarding the "discovery" of radical cationic intermediates in lignin biodegradation by a DSM/Imperial College team and by the Madison, WI, group led by Kent Kirk (*Bio/Technology* 4:1103, Dec. '86).

Without any doubt, Professor Crawford is right in drawing attention to the important contributions made by Kirk's group, most notably discovering the "unique H_2O_2 -requiring oxygenase," or ligninase. In this respect, however, the work of M. H. Gold (Glenn et al., *Biochem. Biophys. Res. Comm.* 114:1077-1083, 1983) should be mentioned also. These investigators independently discovered ligninase (or diaryl-propane oxygenase, as they called it). In fact, results described in this paper—in combination with previous labeling studies by the groups of Kirk, Gold, and Higuchi—led me to conclude that ligninase is best classified as a peroxidase rather than an oxygenase, and that radical cationic intermediates can explain most of the results obtained so far. Moreover, we predicted in our discussion letter (Schoemaker et al., *FEBS Letters* 183:7-12, 1985) that then-unknown ligninase catalyzed reactions like aromatic hydroxylation and "remote" oxidation.

In addition, Kirk et al. have described superoxide anion formation in one specific example (Hammel et al., *J. Biol. Chem.* 260:8348-8353). We have now shown that this oxygen activation is a general phenomenon (Palmer et al., *Phil. Trans. Roy. Soc.*, in press).

I put oxygen activation in its correct historical perspective here because its applications deserve special attention. Due to the very nature of the lignin structure, this activation process is inevitable and might have serious drawbacks. Despite tremendous progress in the last few years, in my opinion the study of lignin biodegradation is still in its infancy. Although competition is healthy, the complexity of the problem calls for international cooperation.

Dr. H. E. Schoemaker
Bio-Organic Chemistry Section
DSM Research
P.O. Box 18, 6160 MD GELEEN
The Netherlands

IMPROVED SOMATIC EMBRYO RECOVERY

To the editor:

With the advent of somatic embryogenesis and polyembryogenesis in commercially important softwood trees (e.g. *Bio/Technology* 5:147-151, Feb. '87), our attention has now focused on ways to improve the recovery of somatic embryos from cell suspension cultures. We knew from the acetocarmine reactivity of

proembryonal cell cultures that the *potential* for recovery was very great—more than 1,000 from 1.0 ml packed cell volume (PCV). In reality, however, very few (less than 2) transplantable embryos would be recovered per ml PCV.

We now have evidence that, based on the transfer of cell suspensions to a simple support, e.g. folded cheese cloth in a Petri dish containing medium (see Figure 1), and prior pretreatment of embryonal cell masses with abscisic acid (4 to 11 μ M), we can now recover a first crop averaging 25 plantlets per ml of cell suspension culture (see Figure 2). With further improvement in this process, we believe that the potential exists for additional orders of magnitude increase in plantlet recovery.

These data come from the work of Michel Boulay, Pramod K. Gupta, and myself. Our results indicate that the production, storage (cryopreservation), and conversion of embryonal cell suspensions without callus formation will someday be useful as a complementary technology to seed orchards in clonal forestry. In seed orchards, trees are sometimes grafted, and seed production is erratic and at the mercy of diseases, insects, and environmental vagaries.

We anticipate that with continued work other researchers will soon be able to field-test these progeny for their trueness-to-type, genetic variation, and genotype/environmental in-

Somatic embryos transferred to a cheese-cloth support in a Petri dish containing medium (Top) show improved ability to regenerate into plantlets (Bottom).