L-Tryptophan Puzzle Takes New Twist

Last week Michael Osterholm admitted publicly what teams of federal investigators have known for months: batches of the dietary supplement L-tryptophan that have been implicated in a mysterious disease were produced by a genetically engineered organism. Osterholm, an epidemiologist at the Minnesota Department of Health, never said that gene splicing was to blame for the outbreak of eosinophilia-myalgia syndrome, or EMS, which to date has claimed 27 lives and affected 1535 others. He simply said it was a hypothesis that must be investigated.

But his carefully crafted words, first published in an interview with *Newsday*, engendered a spate of newspaper headlines about genetic engineering gone awry and stirred up quite a ruckus at the Food and Drug Administration (FDA), where officials were apparently hoping to keep the recombinant link quiet until they could determine whether it in fact did play a role in the outbreak. Osterholm is the first to admit that the question is not at all clear.

Sam Page, chief of the natural products and instrumentation branch at FDA, immediately blasted Osterholm for "propagating hysteria." Page told *Science*: "The whole question, Is there any relation to genetic engineering? is premature—especially given the impact on the industry." Page concedes, however, that FDA and the drug's manufacturer are looking into the possiblity that the gene-spliced bug is somehow to blame.

Osterholm, for his part, seems somewhat bewildered about the furor his remarks have caused. "Anyone who looks at the data comes to the same conclusion," he says—namely, that the genetically engineered strain may be involved. "I think FDA doesn't want it to be the case because of the implications for the agency. But that should not blind them to the possiblity, which is all I am suggesting."

Last November Osterholm's group and another in New Mexico linked the strange outbreak of EMS to L-tryptophan, which, before it was recalled, was sold in health food stores and was widely used for such ailments as insomnia and depression. In much of Europe, by contrast, the amino acid is regulated as a prescription drug and is much more difficult to obtain. To date, the only deaths from EMS have occurred in the United States. And just 2 weeks ago, in an article in the *New England Journal of Medicine*, Osterholm and researchers from the Mayo Clinic traced the outbreak back to tryptophan made by just one of the six Japanese manufacturers—Showa Denko K.K.—and to specific lots made between October 1988 and June 1989 (*Science*, 10 August, p. 619).

Without ever mentioning genetic engineering, they noted that this coincides with Showa Denko's introduction of a new strain of bacillus, Strain V, used to produce the tryptophan, as well as a couple of other manufacturing changes, such as a reduction in the amount of activated carbon used for purification.

What's more, the Minnesota researchers then identifed a chemical constituent, which they dubbed peak E, in these batches of tryptophan that seems to be the culprit. They can't say for sure that peak E is the causative agent, but the evidence is "very strong," says Osterholm, who adds that of the 30 different contaminants they found, only peak E was strongly linked to the disease. Page agrees: "It's what we're going after." Osterholm and his colleagues caution, however, that peak E could nonetheless be a surrogate for the real etiologic agent.

Almost as soon as they found peak E—and several months before publication—Osterholm and his colleagues told Page and the other researchers at FDA and the Centers for Disease Control about it. Since then, both groups have been furiously trying to work out its chemical structure, which proved to be a bear, says Osterholm. Both groups say they have just succeeded, though neither will reveal anything about the compound except that it is a dimer—essentially, two tryptophan molecules linked together. Both groups are submitting papers for publication.

With the structure in hand, researchers can now begin to nail down whether peak E is indeed the causative agent, and if so, where it came from. And that is where genetic engineering may come in, says Osterholm. "There was an effort [at Showa Denko] to turn the bacteria into an L-tryptophan powerhouse," says Osterholm, who notes that Strain V was engineered to crank out more of two intermediates involved in tryptophan biosynthesis, and thus more tryptophan as well. Showa Denko has described the details of the gene splicing to the U.S. researchers and is working closely with the investigation.

The gene splicing may have nothing to do with the creation of peak E, says Osterholm—the dimer could be a breakdown product of tryptophan, for example, arising from other changes in the manufacturing process. But to him, the obvious question is whether the modified organism was somehow inadvertently programmed to produce more of the double tryptophan.

"The key fact, we believe, is it is a double hit," says Osterholm, referring to both the introduction of Strain V and the reduction in carbon purification. The upshot, he suspects, is that the modified strain may have made more of peak E, which was not removed from the sample because of the change in purification. Combine that with a hefty dose of these capsules from the health food store and you could have a formula for disaster.

"There are too many loose ends to justify this loose talk to the press," retorts Page. First of all, he reiterates, there is no proof that peak E is the causative agent. Nor is there any proof that that Strain V actually produces peak E. In fact, he says he has evidence suggesting that peak E may arise from a chemical reaction, not a biological one. He expects to sort it out within a couple of months, as does Osterholm, who is beginning studies to see whether the modified organism produces peak E.

■ LESLIE ROBERTS

A Go-Ahead for Mount Graham?

To the outrage of environmental activists and the delight of University of Arizona astronomers, the U.S. Forest Service on 23 August okayed the construction of three telescopes atop Arizona's Mount Graham—a 10,700-foot peak whose summit has been designated a critical habitat for the endangered Mount Graham red squirrel.

The ruling is only the latest episode in an ongoing battle between the astronomers and environmentists, who bitterly dispute the claim that the telescopes will not harm the squirrel (*Science*, 22 June, p. 1479).

The environmentalists had been pinning their hopes on two studies this summer by the General Accounting Office and an interagency biology team, both of which calied for more research on the squirrel before the observatory is allowed. But the Justice Department ruled last week that further study was precluded by the Arizona-Idaho Conservation Act, an exemption to certain environmental requirements that the university lobbied through Congress in 1988. Thus, the Forest Services' go-ahead.

The university immediately prepared to start construction. But as *Science* went to press, environmental groups were asking for an injunction in federal court.

■ M. MITCHELL WALDROP