because changes in the variable region can be introduced more quickly. "By doing site-directed mutagenesis in the variable region, you might be able to create new antibodies," concurs Heyneker. "But you have to develop very nifty screens to fish out the mutations."

Single-chain molecules are also easier to purify to homogeneity, so analysis of their structure via x-ray crystallography will be easier. Ladner adds that generalizing the technology across different antibodies will be much simpler than designing the original single-chain.

Genentech's Wetzel estimates that in order to properly position the antibody fragments, the protein linker needs to span about 30–40Å. This would require some 15–25 amino acids, he says, but the exact number would depend on the extent to which the linker takes on a secondary structure of its own. There are potential advantages and disadvantages of each such linker configuration, he explains, so it is difficult to predict which will work best. One strategy would be to give the linker as little regular secondary structure as possible by using amino acids that are predicted to form a random coil. This would probably not interfere with the immunoglobin's folding, but it might be susceptible to proteolysis. Alternatively, if researchers gave it more secondary structure, the linker might prove more stable. However, this structure—especially if it was a beta-sheet—could interfere with the formation of the twisted beta-barrel structures of the heavy and light chains. A preferable choice for the linker's structure, Wetzel argues, might be an alpha-helix, although this would require more amino acids than the others.

There are other concerns as well. According to Wetzel, a hydrophilic linker would probably interfere least with the heavy and light chain regions as they try to fold. On the other hand, such a linker might be subject to proteolysis. "One is going to have to find the happy medium empirically," Wetzel concludes. "It might even be best to use a random method and then screen for success."

The presence of a linker molecule could give single-chain antibodies a lot of flexibility. For example, Heyneker notes that researchers could incorporate a cysteine residue into this spacer that would facilitate a chemical reaction with an immobilized support, causing the antibody to stick. "The key," he says, "is if you can do it economically." Eventually, Heyneker adds, the linker portion could be endowed with toxin properties, so such a construct could be used in cancer therapy. Alternatively, the linker could be designed in such a way that it allowed an anti-cancer agent to bind to it without obstructing the nearby binding site.

Celltech is emphasizing the cancer applications of its recombinant antibodies, through its agreement with American Cyanamid's Lederle Laboratories (Wayne, NJ). According to Celltech's marketing director, Nic Holladay, under the two-year contract Lederle made one £2.5-million R&D payment last spring and will owe an equal sum at the same time next year. He says the work remains early-stage, focusing on various cancer and solid tumor imaging agents; eventually the scientists will address therapeutics that take advantage of an antibody's binding fragment attached to an anti-cancer agent.

According to Holladay, animal trials could begin by the end of 1987; this could give researchers their first real information on how these molecules act in the body. Sherie L. Morrison of the College of Physicians and Surgeons of Columbia University (New York, NY) thinks they could have some real advantages: "My guess would be that a variable region alone would be less immunogenic than the whole molecule," she says. Morrison's own work centers on the creation of human-mouse hybrid monoclonal antibodies (see *Bio/Technology* **4**:392, May '86). Here the idea is to combine human constant regions (for less antigenicity *in vivo*) and mouse variable regions (for antigen binding).

Heyneker isn't so sure that the smaller, single-chain antibodies will solve all the antigenicity problems. "Single-chain antibodies are probably pretty antigenic because of the peptide linker," he says.

Ladner from Genex points out that there will always remain situations where researchers will prefer to use the whole antibody molecule. For example, the constant region contains binding sites for other components of the immune system, such as white blood cells. Thus this portion can be important for recruiting the body's natural defense mechanisms in therapeutic applications. In fact, at least one company, Immunetech Pharmaceuticals (San Diego, CA), emphasizes harnessing the active sites on the *constant* region as a way to control the body's immune system.

But single-chain antibodes could represent the most promising strategy of all. Concludes Morrison: "When all you need is antigen binding, then that might be the right approach."

—Arthur Klausner

BIO-CONTROL

FINNS FIND FUNGAL FIGHTERS

JOKIOINEN, Finland—Well established as prime antibiotic producers, streptomycetes are finding new applications as biological control agents.

This further utility came to light during studies here at the Agricultural Research Centre into *Sphagnum fuscum* peat. Freshly dug, this light-colored material is the most common substrate for greenhouse cultivation in Finland. It has the marked advantage over soil of being entirely free of plant pests and pathogens. Several years ago, Risto Tahvonen and his colleagues decided to investigate whether this was a chemical or microbiological phenomenon, and found that the peat's natural microbial flora strongly inhibited the growth of seed- and soil-borne fungal pathogens. Steam sterilization completely abolished this inhibitory effect, which reappeared when they added fresh, non-disinfected peat. *Trichoderma viride* was one potent agonist, but another major contributor came from species of *Streptomyces*, which now have proved particularly convenient for the control of greenhouse pests.

Having identified the most effective *Streptomyces* isolates, Tahvonen has prepared spore suspensions for coating seeds and dipping root cuttings as a means of combating damping off, cucumber root diseases, *Fusarium* wilt on carnations, and *Botrytis* rot on lettuce. Treating cabbage seeds totally abolished seed-borne damping off normally caused by *Alternaria brassicicola*. In other cases, such as cucumber root diseases produced by species of *Fusarium*, *Phomopsis*, and *Pythium*, spraying the soil alone gave satisfactory protection.

On a practical scale, the outstanding success so far has been with cucumbers. Yields have increased by 10 percent, and it has been possible to maintain productive stands until the end of the growing season without needing to replant. Wilt disease in carnations has also been checked so effectively that the area of plants that has had to be destroyed in two-year cultivations has been under 10 percent, compared with 30–40 percent for untreated plants.

—Bernard Dixon