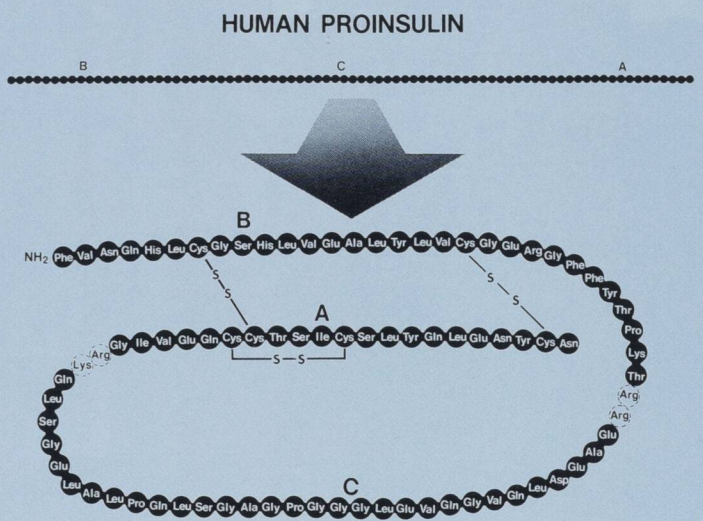
Pursuing the practical payoff

The downstream end of the Narang-Wu insulin project, which involves the production of the hormone for use by diabetics, was transferred in 1982 to Connaught Research Laboratories in Toronto. As Dr. Eric James, head of Connaught's recombinant-DNA laboratory explains, it's a long way from a bacterium that carries a plasmid with the proinsulin sequence to one that produces proinsulin; it is an even longer journey to a bottle of the active hormone on a druggist's shelf. "The modified bacterial strain sent to us by Dr. Wu's lab in Cornell was not designed to produce the proinsulin protein. Instead we used it as a source of the gene." The problem Connaught faced was in the bacterium's defensive enzymes, which break down the proinsulin protein produced by the gene before it can exit from the cell. Proinsulin is, after all, a foreign protein to *E. coli*.

"To get around this, we had to 'mask' the protein — that is, link it up to something the bacterium viewed as part of itself." The 'something' James and his Toronto colleagues used was one of the bug's own enzymes, a large protein called beta-galactosidase. Using genetic engineering techniques, they joined the 287 base-pair sequence of the proinsulin gene to the 3000 base-pair enzyme gene. As they hoped, the bacterial defence system ignored the 'chimeric' protein produced from the large hybrid gene.

To make it easy for Connaught to lop off the unwanted enzyme portion of the protein, the Ottawa-Cornell team built their proinsulin gene with the code for the amino acid methionine appended to the end slated for linkage with the enzyme gene. With this amino acid joining the two parts, it is a relatively simple chemical task to separate them after the hybrid is retrieved from the bacterium. The chemical cyanogen bromide degrades methionine (thereby cleaving the duet) and, luckily, this amino acid does not appear in proinsulin.

"We now have proinsulin from this cleavage," says Dr. James, "and our next step is to carry out the cross-linking reaction that occurs when the linear protein folds over on itself. Lastly, the centre part of the fold will be snipped out using enzymes, and we'll have the active hormone." (see diagram)



Connaught is working hard to produce gram quantities of the hormone by next summer, and hopes to improve the system's efficiency by way of certain modifications. For one thing, the huge enzyme mask — over ten times the size of the proinsulin — represents a lot of waste effort on the part of the biosynthetic system. James says that their research indicates that a fragment of the enzyme molecule, perhaps as little as one quarter of it, will do the needed masking job.

The Toronto team is also looking into the possibility of using other microorganisms — yeast for example — as hosts for the hormone gene. Currently, Connaught is the main supplier of insulin in Canada, obtaining its hormone from the pancreases of hogs and cattle.

Saran Narang leans back in his chair, savoring the memory of that early success. Granted, Gilbert had produced the first proinsulin protein from transformed bacteria, but he had used a cell-derived gene rather than a man-made gene like that of the Ottawa-Cornell team. Other laboratories, notably the pharmaceutical company Eli Lilly, arrived at the hormone by the more onerous procedure of producing the A and B chains separately in bacteria and then joining them chemically. Eli Lilly also showed clearly in a British study last year that human insulin derived from bacteria is effective in treating diabetics.

There were, and are, other problems to solve in getting insulin production geared up to the same technological status as beer-making. It was found, for example, that the bacterium did degrade the proinsulin, and to combat this a bacterial gene for one of its own proteins was spliced into the plasmid just in front of the proinsulin. Now, as Narang's darkened micrographs attest, the breakdown enzymes leave the mosaic protein alone, viewing it as one of their own. Narang placed a bridging stretch of DNA into the gene that makes separation of the two proteins an easy chemical procedure.

Today, though NRC still does research in the area, the downstream, or factory-scale process for producing the vital hormone has been passed over to Canadian industry (see "Pursuing the practical payoff"). As for Saran Narang, he still has an interest in how things are going, but his attention has passed on to other things. The making of human insulin has left him expert with the tools of gene synthesis and he is now turning them to a study of what he calls "jumping genes."

Saran Narang: "Certain genes, more properly called 'transposable elements,' seem capable of ferrying genes back and forth between the chromosomes. Wouldn't it be delightful if, one day, we could use them to reintroduce whatever is missing to a diabetic's pancreas cells . . ."

He pauses, hesitates, and moves to the blackboard, alone despite the company, lost in the new idea.