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Editor's Note

This first number of the 1981 series marks the *Recombinant DNA Technical Bulletin's* fourth year as a publication of the National Institutes of Health. The continued growth of the *Bulletin*, over this period, has necessitated the changes in format evident in Volume 4, Number 1.

As the *Bulletin* begins its fourth year, we would like to take this opportunity to express our appreciation to those who have contributed so generously to the *Recombinant DNA Technical Bulletin.*

Elizabeth Milewski, Ph.D.

Workshop on Recombinant DNA Risk Assessment Pasadena, California

On April 11-12, 1980, the National Institute of Allergy and Infectious Diseases (NIAID), sponsored, in Pasadena, California, a "Workshop on Recombinant DNA Risk Assessment." The Workshop was designed to define the scientific issues and assess the potential risks of (1) possible direct adverse effects of hormone-producing strains of E. coli K-12, and (2) the possible occurrence of autoantibodies or autoreactive cells due to the production of eukaryotic polypeptides (including hormones) by E. coli K-12 should such strains for unexpected reasons colonize higher organisms. In order to address these topics, the meeting brought together scientists from the fields of immunology, endocrinology, physiology, microbiology, infectious diseases and other appropriate disciplines. The information synthesized by the Workshop and Workshop recommendations to NIAID will be used to implement the National Institutes of Health program to assess the risks of recombinant DNA.

The Recombinant DNA Technical Bulletin will compile and publish, over several issues, data presented at or generated by the Pasadena Workshop.

This issue of the Bulletin publishes two presentations from the "Peptide and Hormone Workshops."

Expression of Active Polypeptides in E. coli

Walter Gilbert
Harvard University
Cambridge, Massachusetts 02138

I would like to discuss two topics: (1) how proteins made in bacteria might enter the environment and (2) I would like to address risk by formulating possible hazards associated with a systemic infection by *E. coli* producing insulin or interferon.

There are several methods of engineering bacteria to produce eukaryotic proteins. One of these techniques, pioneered by Genentech, Inc., produces insulin by combining purified insulin A chains produced by one strain of bacteria with insulin B chains produced by a second strain of bacteria. On its face, this procedure probably does not present any particular hazard, as an active hormone or polypeptide is not produced.

In a second technique, a fusion protein of proinsulin or preproinsulin linked to penicillinase is produced. The fusion product can be engineered to be secreted from the bacterial cytoplasm into the periplasmic space. Many bacterial proteins are secreted from the bacterial cytoplasm into the periplasm: these proteins are synthesized as precursor proteins with a hydrophobic leader sequence at the amino-terminal end.

In order to study transport of insulin or proinsulin out of the cytoplasm into the periplasm, Karen Talmadge of my laboratory created a set of plasmids in which the gene coding for insulin or proinsulin was linked to various positions in the hydrophobic leader sequence of penicillinase. A striking observation from this work is that when most of the proinsulin leader sequence or a complete penicillinase leader sequence is present, 90% of the protein produced is transported to the periplasm. When codons for charged amino acids appear after the hydrophobic leader sequence, only 50% of the product is transported into the periplasm. Proteins with "damaged" leader sequences are not transported. Thus a hydrophobic leader sequence is necessary to transport proinsulin into the periplasm; a eukaryotic as well as a bacterial leader sequence suffices.

As the eukaryotic leader sequence effects transport into the periplasm, one might ask whether the bacterial host will "clip" off the proinsulin leader sequence. The data show that the bacteria correctly cleave off the eukaryotic leader sequence. Thus a complete, functional proinsulin structure would be made in bacteria. We believe that the same phenomena is occurring in bacteria producing leukocyte interferon. There is a presequence on leukocyte interferon, and some of the biologically active interferon produced in Charles Weissman's laboratory may be matured.

How much insulin is actually being produced? Our original strain produced about 100 molecules of proinsulin per cell. Our best producer strain currently produces about 6,000 molecules of proinsulin per cell. One would hope to eventually construct *E. coli* strains that will devote a reasonable fraction of cellular protein synthesis to production of an active protein. At best, one might succeed in constructing a strain producing about 10^6 molecules (of whatever you hope to make) per cell per generation (about 30 percent of the possible protein initiations). For proinsulin, this maximum production would be about five milligrams per gram of cells (5×10^{11} *E. coli* cells). For interferon, maximum production would be about fifteen milligrams per gram of cells.

Would cells producing this level of material constitute a hazard should the gastrointestional tract be colonized by hormone-producing *E. coli*? In order to determine the amount of insulin produced

we will use the figure given this morning, 2×10^9, as the number of *E. coli* in the gastrointestional tract per day; bacteria duplicate about every 12 hours in the tract.

In the case of insulin, 2×10^9 bacteria will produce 25 gamma of proinsulin. Twenty-five gamma of proinsulin is equivalent to one-half unit of insulin activity. At this level, a change in blood sugar level is not perceptible.

Using interferon in this example, 75 gamma of material would be produced in an individual totally colonized by interferon-producing *E. coli.* Interferon is far more active biologically than insulin. A dose of insulin is on the order of a milligram of material; a dose of interferon is of the order of ten gamma.

I regard these calculations as being "worst case." The bacteria we now have in the laboratory make one one-thousandth of the amount of insulin used in the calculation above. The same is true of interferon; laboratory strains produce on the order of 10^6 units per liter of culture. In order to reach the above estimate of maximum amounts, one must assume that the gut is totally colonized by *E. coli* producing active-polypeptides, moreover these *E. coli* must colonize the gut for long periods. In addition, the bacteria must maintain the ability to devote one-third of their protein-producing capacity to the synthesis of active polypeptides. It is probable that most of the strains designed for commercial purposes will be temperature sensitive or the promoter will be under other control; these strains will probably not, except in ideal conditions, produce appreciable amounts of the active polypeptide.

Furthermore, there is a high level of proteolytic activity in the gut, which will digest most hormones. In one experiment two hundred and fifty milligrams of insulin, put into the gut, had no effect on the volunteer's blood sugar; all the insulin was destroyed.

Discussion

Dr. Martin: Can these arguments be applied to other peptide hormones?

Dr. Sherwood: The levels of polypeptide hormones in the blood are in the range of 10^{10} molar to 10^{11} molar. The normal circulating level of growth hormone is one to two nanograms per milliliter. I calculated the increase in the blood level of human growth hormone in a systemic *E. coli* infection assuming that all of the *E. coli*-produced growth hormone would be released into the blood. In a case of *E. coli* sepsis with 40 to 50 organisms per milliliter (actually 10 organisms per milliliter is the more normal value), a quarter of a picogram of additional hormone would be produced. *E. coli* sepsis would be a far more significant clinical problem. Insulin production, because of the effects of prolonged hypoglycemia, might be the most life-threatening model to consider.

Dr. Goldberg: How about interferon?

Dr. Gilbert: The effects of interferon are discussed on several levels in terms of effects on the body. Currently about ten gamma are administered through injection. Doses of about 50 gamma probably can be tolerated without side effects. Above that, serious effects on the blood forming system might appear.

Dr. Goldberg: One might, however, consider the effect of high local concentrations as might occur in an abcess.

Dr. Gottesman: Is there any information available on whether fusion proteins, improperly cleaved by the host bacterium, might act as a competitive inhibitor or blocker of normal insulin? Could such an improperly processed protein accumulate in the serum?

Dr. Gilbert: I don't know the answer to that question. The fused proteins, at least superficially, are antigens of a different nature than the original proteins.

Bacterial Factor with Gonadotropin Activity

S.S. Koide
Center for Biomedical Research,
Population Council,
The Rockefeller University
New York, New York 10021

T. Maruo
School of Medicine
Kobe University
Kobe, Japan

Production of a substance resembling chorionic gonadotropin (CG) by microorganisms was reported by several investigators: Livingston and Livingston (1974); Cohen and Strampp (1976); Acevedo *et al.* (1978, 1979); Slifkin *et al.* (1979); Richert and Ryan (1977a); Affronti *et al.* (1977a, b) and Koide *et al.* (1980).

Identification of the substance as CG was based on hemagglutination inhibition assay, radioimmunoassay, radioreceptor assay methods and immuno-cytochemical techniques. The assay methods are subject to errors by interfering substances such as proteases and non-gonadotropin immunoreactive substances