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BIOTECHNOLOGY: APPLICATION OF GENETIC ENGINEERING.

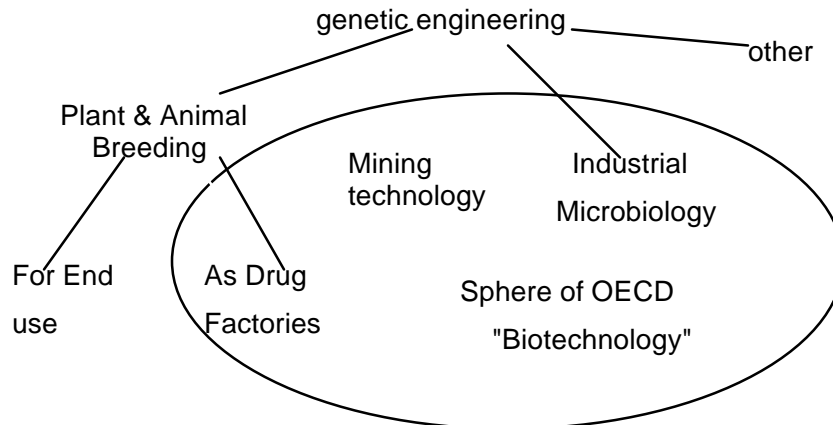
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Introduction

"We wish to suggest a structure of the salt of deoxyribonucleic acid (DNA). This structure has novel features which are of considerable biological interest" Thus went the opening sentence of the most famous paper ever to appear in *Nature* (the world's leading scientific journal.) The date was 25 April 1953. In it Francis Crick and James Watson first described the double helix structure of DNA.

This scientific discovery made possible the technology of genetic engineering which has had wide ranging applications in animal and plant breeding on the one hand and Industrial Microbiology on the other.

Fig. 405 Biotechnology and Some Fields of Application of Genetic Engineering



Genetic engineering

The term genetic engineering has been applied to various high-tech aspects of biological technology. *All involve the removal of a gene or a segment of DNA from one cell and its insertion into another.*¹ A subset of techniques, concerned with the introduction of genes from other species, through genetic engineering rather than hybridization produces transgenic animals, plants and other organisms and has been called transgenesis². Genetic engineering is applied in therapeutics; in plant and animal breeding for human use as food, textiles, building materials; and in biotechnology. Insertion techniques include tungsten bullets, syringes, vector micro-organisms and a test tube reaction with a DNA salt.

Biotechnology

The original meaning of the term "biotechnology", and the only one in the 1972 edition of the Oxford Dictionary, is "the branch of technology concerned with the development and exploitation of machines in relation to the various needs of human beings". It included tools for medical research. To-day we would call that bioengineering.

Within the OECD³, the term "biotechnology" is now limited to "the application of scientific and engineering principles to the *processing of materials by biological agents to provide goods and services*."⁴ The biological agents referred to include microorganisms such as bacteria and yeasts used in fermentation; specially bred mammals such as cows, pigs and mice whose milk is designed to contain concentrations of valuable drugs; and specially bred plants whose oils and proteins may likewise contain concentrations of valuable drugs.. Biotechnology involves both cloning known genes and creating new combinations (recombinant DNA)-- or novel biopolymers -- by moving genes from one cell to another by means of genetic engineering. Recombinant DNA technology allows the creation of life forms never seen before.

Some authors extend the meaning of the term "biotechnology" to cover all the new reproductive technologies (both human and animal) as well as those concerned with the biological processing of material. Others (e.g. Australian Biotechnology Association⁵) exclude the processing carried out by whole animals. I shall adopt the OECD definition in this chapter.

Animal breeding

Traditional methods of animal breeding relied upon the selection of parents with the desired traits and had, as their targeted end product, an improved breed of beef, dairy cow, swine etc. Before the introduction of genetic engineering into animal breeding, traditional methods had already been transformed through the development of new technologies. The most important innovations during this period were the introduction of commercially available Artificial Insemination in the 1950s, the introduction of superovulation and embryo transfer in the mid 70s, and subsequent successful cloning⁶.

Another transformation of animal breeding is now coming about with the introduction of various types of transgenic animal. In Alberta⁷ cows have been genetically altered in order to induce them to turn on the interferon in the liver before being challenged by viral disease; transgenic mice are being created which grow to very large size, or are very susceptible to cancer and thus suitable for use as research animals. Researchers hope to turn other cows, pigs and mice into potential drug factories by genetically altering the composition of their milk; this work would be included in the OECD category of biotechnology.

Plant breeding

The technological triumphs of "The Green Revolution" were the product of conventional plant breeding by hybridization, cloning etc.. The "biological revolution" associated with genetic engineering is having an even greater impact on plant breeding than on animal breeding. We can make the same distinction as we did for animals between plants being bred for food and plants being bred to act as processors for the production of valuable chemicals. The latter category would be classed as "biotechnology".

Genetically engineered food plants

Marketing started in February 1994 of the FlavrSavr tomato, bred by Calgene to switch off the gene that starts the softening process. This is done by removing the gene and reinserting it backward; it is therefore not a transgenic product. The Chinese have reported success with transgenic tomatoes designed to fix nitrogen by incorporating genes from the leguminosae. The marketing in Canada of transgenic potatoes developed by Monsanto, under the trade name NewLeaf, started in November 1995. These potatoes contain a bacterial gene that is deadly to insects. The marketing was done without the knowledge of environmental regulators or consumers; they were simply mixed in with other russet potatoes. One's reluctance to eat any genetically altered product may be tempered by the knowledge that the insect-repellent potatoes are unlikely to have been heavily sprayed with biocides.

Native farmers in West Africa discovered the resistance of varieties of cowpeas to insects. Attempts are now being made to transfer genes from the cowpeas into sweet potatoes. Another new breed of insect-repellent potato, called Desirée, contains a gene transferred from peas.

The companies that market herbicides such as Roundup (Monsanto) have created Roundup herbicide-resistant soya beans by adding genes from fungi, bacteria and viruses. The first commercial crops were harvested in 1996 and entered the food distribution system⁸. Trials of Roundup resistant Canola took place in Alberta in 1993.^{9,10} "If herbicide resistant Canola pushes average production up, farmers will have to use it to stay competitive." Monsanto claims that farmers will use less herbicide, helping the environment. Critics say that this new technology simply reinforces the industrial model of agriculture and that more traditional methods of crop rotation would have been preferable.¹¹ Biological pest control, whose efficacy is now being demonstrated at the Muttart Conservatory in Edmonton, is an alternative technology to be explored. A serious concern is the possibility that herbicide resistance can be transferred to weeds by bacterial vectors. There is some evidence that this has occurred.

Transgenic plants as chemical factories

The Alberta Research Council believes that genetically engineered new crops will act as cheap solar-powered, non-polluting chemical factories. Research at the University of Calgary led by Dr. Maurice Moloney is concentrated on Canola as a suitable plant for this purpose. Moloney, in a joint venture with the University, has set up a company called SemBioSys Genetics Inc which is working on the production of industrial enzymes, pollution-fighting proteins and enhanced animal feed. One of the most interesting applications, because it involved the insertion of a human gene into the Canola plant, was to produce interleukin-1. The vector for this is *Agrobacterium* which is introduced into a Canola cutting carrying a piece of human DNA spliced to oleosin DNA (a naturally occurring protein which is unusual in being oleophilic rather than hydrophilic) so that when the product is harvested it can be collected from the oil phase. In 1995 Dr. Moloney and his team harvested 2.5 t of Canola seed containing hirudin, a blood anticoagulant made with a gene from the medicinal leech¹².

Industrial microbiology and Biotechnology

The new technologies of instrumental control and genetic engineering have radically changed the old ways of Industrial Microbiology, an industry which has great antiquity.

For millennia people practised this type of biotechnology as a craft. Everyone is familiar with the fermentation of grain using yeasts to produce bread and beer. Milk products from cows, mares, sheep and goats are also fermented for drinks and foods such as yoghurt and cheese using bacteria, yeasts and moulds.. Bean products are fermented with yeasts and moulds to produce foods such as miso, soya sauce and tempeh. Indeed, almost any vegetable product is fermented in one part of the world or another, from the hearts of palm trees to potatoes.

What has been called the "biorevolution" is the application of theoretical knowledge to what previously was a craft. To that extent it is an exemplar of post industrial technology. During the last century or so biotechnology has been consciously employed in waste management, especially the treatment of sewage by bacteria, but more recently in the treatment of solid waste. However, what is most commercially significant is that a large sector of the pharmaceutical industry uses biofermentation, in many cases using genetically altered organisms, or monoclonal antibodies, for the production of antibiotics and other valuable chemicals.

Monoclonal antibodies

Cell fusion occurs naturally in some cells when they are attacked by a virus. Promoted artificially it produces a heterokaryon of two or more nuclei with a single cytoplasm. By combining a cancerous cell with a cell producing a desirable substance such as insulin or interferon, large quantities of these chemicals can be produced for medical prophylaxis and treatment. For instance a monoclonal antibody (a protein used by the body to fight disease) can be "produced by fusing a myeloma (a type of cancer) cell with cells of the spleen of an animal which has been immunized with a specific type of bacteria or fungi."¹³

The Nobel Prize was awarded to George Köhler¹⁴ and César Milstein for the development of monoclonal antibodies at Cambridge in the 1970s.

Most of the enzymes, antibiotics, vaccines and hormones that are applied in animal husbandry and cultivation are either already the product of such techniques or are targets for processes of that kind. Nineteen biotechnology derived drugs were approved in the USA between 1982 and 1991 but it is expected that over 100 will be approved by 1996.¹⁵

"Monoclonal antibodies are becoming increasingly important tools in diagnosing disease. The range of medical uses includes pregnancy testing and cancer testing as well as diagnosis of viral gastroenteritis, hepatitis B, cystic fibrosis and Sexually Transmitted Diseases."¹⁶ Tests based on monoclonal antibodies can be done in minutes and often require only a single drop of blood.

Transgenic microbiology

Just as in animal and plant breeding, many of the new developments in what used to be called industrial microbiology result from the use of genetically altered bacteria to produce new products -- mainly proteins. A short description of this activity is "DNA makes RNA makes Protein makes money." Enzymes used in food

processing produced in this manner include amylase, catalase and lactase which are used to produce a wide range of products including bread, baby foods, sugar, fruit juices, soft drinks, and corn syrup¹⁷.

Genetic engineering, the creation to order of genetically engineered organisms, has immense possibility for private profit. As a result, serious conflicts of interest have arisen in universities. It has been said that, in the USA, biotechnology has joined basketball as an important source of educational cash.

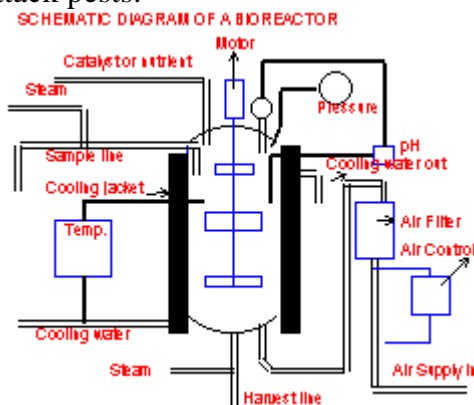
As an example of the new biotechnology, Dutch scientists have used genetically modified bacteria to convert large amounts of ammonia from pig manure into lysine (a protein that can be fed back to pigs).

Recombinant DNA methods are used where the genetic sequences are well understood.¹⁸ Gene machines are able to synthesize specified short sequences of single strand DNA under control of a microprocessor.

"With comparatively simple laboratory techniques involving enzymes obtained from microorganisms to cut DNA molecules into a number of fragments and ligases to splice or rejoin different fragments, recombinant DNA can be obtained which is introduced into the protoplast by means of a vector or carrier DNA molecule; lately by the use of tungsten bullets to fire a plasmid into a cell (Fig. 162)¹⁹". All kinds of therapeutic proteins and growth hormones have been produced in this way and other products, such as a vaccine for Hepatitis B, are likely to follow. In Alberta, researchers are working on a biochemical surfactant for oil recovery.

Modern Bioreactors

The large scale production of many of these biological substances takes place in vessels traditionally called fermenters. The term "bioreactor" would be more appropriate. They may have a capacity of thousands of litres. The Alberta Research Council has some of the largest experimental fermenters in North America. They have one of 15 000 L, 5 of 7 000 and others of smaller capacity. They are used, not only for the fermentation of liquids in the conventional sense, but for the growing of bacteria or even of nematodes on a very large scale. The nematodes are to be released in fields to attack pests.



Fermentors, like barrels and tubs, have been used for centuries. Where we see a major technological change is in the instrumentation and control. Instrumentation is a vital technological contribution to the advancement of science. In this case instrumentation has revolutionized the art of fermentation and made it into a science.

The adjacent schematic shows a typical fermentor or batch reactor. It is a vessel in which the nutrient medium and a biological catalyst are mixed and given a favourable environment in which to react. The temperature is carefully monitored and controlled by a valve regulating cold water entry to the cooling jacket -- since these are

exothermic metabolic reactions. The pH is monitored and controls the pump from the acid or base feed, depending on the needs of the reaction. Some processes are aerobic (require oxygen) in which case filtered air, sometimes enriched with oxygen, is fed into the base of the reactor under constant monitoring and control. The entire contents of the reactor are kept in motion by a motor-driven set of paddles. All control and data are under the command of a computer program. It has been said that the computer has revolutionized the fermentation industry.

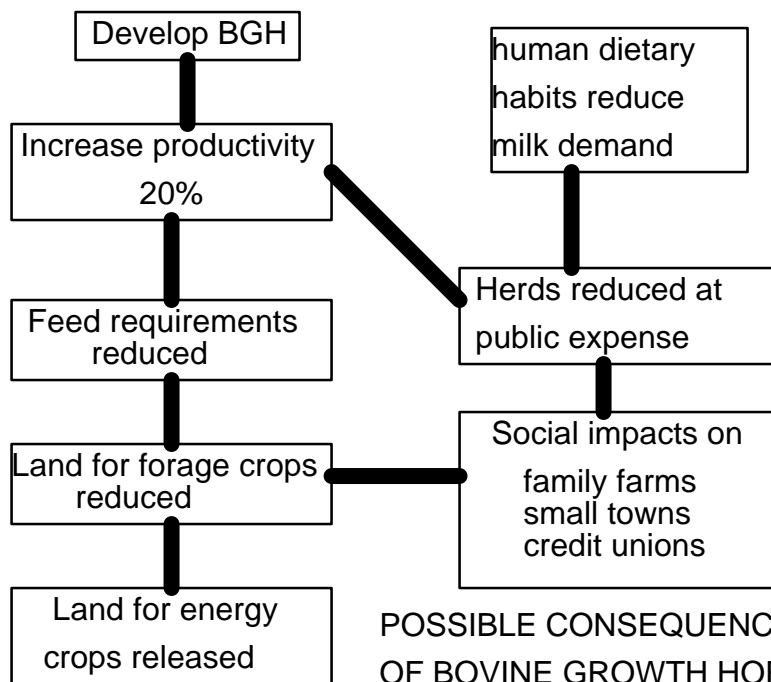
There is a line at the top of the reactor through which samples can be removed for biological and chemical analysis. When the batch is complete, it is harvested through a line at the bottom of the reactor and the whole apparatus is sterilized with steam before a new batch is prepared. If the previous historical model of industrial process technology is followed, we may see continuous processes invented to replace batch methods.

Products of transgenic industrial microbiology

In this section I discuss a few of the products of the new technologies, such as hormones and genetically altered bacilli, which appear to have important social consequences.

Bovine Somatotropin (BST)

Bovine Growth Hormone is an example that is causing ethical concern.



The US Food and Drug Administration issued its registration for BST in November 1993. Some possible consequences of its introduction are shown on the adjacent chart. It is worth recalling that during the period 1950-1980 the productivity of Holstein cattle doubled as a result of conventional breeding methods. During this period seventy percent of the dairy farms in the U.S.A. abandoned milk production. University of Alberta research

suggests that use of the bovine somatotropin hormone will shorten the productive life of the cows so capital costs may rise and partially offset gains for the dairy farmer²⁰.

Human Growth Hormone

Human growth hormone may cause even more concern since information was published suggesting that it could reverse the effects of aging²¹. However, there are pronounced side effects and there is no evidence that it improves life span. It just makes people leaner and more muscular. There is a grave danger of misuse because of the atavistic social importance given to height in our culture.

Ice-Minus

Deletion of individual genes in bacteria has eliminated the protein that causes nucleation of ice. Bacteria thus altered have been sprayed on strawberry crops (1986)²². Another example of a deleted gene is the pseudo-rabies livestock virus. Gene deletion is considered a safe procedure because it happens all the time by chance in nature. The results are usually disadvantageous - if they were not, you would find natural populations with the deleted gene. It is strange that one of the arguments against the use of the ice-minus *Pseudomonas* was "What if it had an adverse effect on honey bees?" The question seems not to have been asked about furfuran spray which is *known* to kill them.

Anthropological research reported by Usher Fleising²³ suggests that a good deal of the objection to the use of the ice-minus bacteria on strawberry crops came from growers in the area who felt it would give an advantage to other regions by extending their growing season. An extensive study of public hearings on technological risk in biotechnology and indeed on technology in general suggests that when the direct interests of a group are at stake no amount of education will have an effect on them. Fleising observes that "The assertion that the key to public knowledge and acceptance of biotechnology is a matter of education is a false orientation".

Mining technology

A less well-known application of microbiology (not involving genetic engineering) is for biohydrometallurgy in the field of mining technology. I believe this process falls within the OECD definition of biotechnology.

Metal sulphides, when exposed to oxygen, are oxidized into metal sulphates and sulphuric acid. The oxidation rate can be accelerated from half a million to one million times by *Thiobacillus ferrooxidans*. In particular this acidophyllic sulphide-oxidizing bacterium enormously accelerates the oxidation of copper sulphide to copper sulphate and ferrous iron to ferric iron -- the potent leaching agent that extracts the metals.

By the mid 1980s the copper industry in the US was on its last legs: low grade ore, low prices and regulations on sulfur dioxide emissions crippled productivity. Now 30% of the copper produced is extracted biologically.

Ethics of genetic technology

Genetic technology allows one to cross all species boundaries and even the boundaries of the plant and animal kingdoms. Jeremy Rifkin calls this work Algeny - by analogy with alchemy, the transmutation of elements. His objection is metaphysical

"The sacred unit used to be the organism, now the sacred unit is the gene ... so we are witnessing a new form of the desacralization of life²⁴." He fears that the proliferation of genetic technology may lead to a view of life as the product of the laboratory and the market place. The fact is that the boundaries of species were crossed long before the intervention of humans, and are being crossed all the time as microorganisms vector pieces of DNA from one organism to another. If Lyn Margulis is right "ten percent of our body weight is bacterial [in its evolutionary origins], and it's just foolish to ignore that."²⁵

Every technological innovation changes the balance of power. Genetic technology may concentrate too much power in certain hands. It adds fuel to the concern about genetic diversity. Already there are only about 8 or 9 breeders of egg-producing chickens in the world.

Rifkin believes that the work should be discontinued. He "doesn't know anyone smart enough or wise enough to design new plants and animals."

There are many molecular biologists concerned about, but not deterred from, research in this field, in spite of the fact that there is an extraordinary cancer rate in genetic engineers at the Pasteur Institute²⁶. David Baltimore's theory was that engineered bacteria carrying the "excess baggage" of foreign DNA in a plasmid were unlikely to spread when forced to compete with native bacteria. Later research²⁷ showed that bacteria may not only tolerate, but even benefit from, the presence of the extra genetic material. If protective genes are inserted into plants, what is to stop them being transferred by viruses to weeds?

The scientific community took the lead in regulating itself as a result of the Berg letter in 1970 followed by the Asilomar Guidelines. They established a Recombinant Advisory Committee composed of scientists, ethicists and philosophers. Since then governments have taken over responsibility from the scientists. In the USA a proliferating bureaucracy has placed the responsibility in a bewildering network of agencies: EPA, FDA, USDA, OSHA. Nets have holes.

As a result of concerns about health, ethics and the environment, an equally bewildering constellation of protest groups has arisen. In addition to Rifkin's metaphysical objections, the following problems have been identified by one group or another:

- the danger that herbicide resistance could be transferred from crops to weeds;
- the danger that the pharmaceutical properties of specially bred plants could enter the food chain;
- the lack of labels on bio-engineered food, denying consumers a legitimate choice;
- the likelihood of new allergens and toxins appearing in the food chain;
- the certainty of unexpected consequences.

With respect to the transfer of unwanted genetic modifications into food plants, Dr. Moloney, in an interview "acknowledge[d] the risk, but says there are several ways of tackling the problem. Crops can be isolated and mechanisms put in place to trace the movement of the seed through the crushing and processing. There are 'extremely sensitive tests' available to detect contamination."²⁸ He thought that there should be no risk whatsoever that "these things" would end up contaminating the food supply.

In a public meeting in Calgary, Dr. Moloney said he had no problem with labelling whole foods such as tomatoes as genetically engineered, because they're safe. "There's no apology to make", he is reported as saying.²⁹

There is, however, published evidence that toxins may enter the food chain through food supplements prepared with biotechnology. In 1989 there was an outbreak of poisoning in the USA traced to tryptophan manufactured by Showa Denko K.K. in Japan. It is reported that 37 people died and 1500 were disabled as a result.³⁰ This seems to have been an isolated case. However, if no further unexpected consequences turn up, it will be the first time in the history of technology.

Review questions

1. What is a transgenic animal? What might you do with a transgenic mouse?
2. Why might some people refuse to eat a NewLeaf potato yet enjoy a FlavrSavr tomato?
3. Give three examples of the pre-industrial use of microorganisms in the preparation of food and drink?
5. In what sense has industrial microbiology gone through a revolution?
6. What role do bacteria have in mining technology?
7. What sort of products would be made from genetically altered bacteria?
8. What ethical problems do you anticipate from the marketing of bacterially synthesized human growth hormone?
9. What are the principal safety and ethical issues arising out of research into genetically engineered plants and animals?

¹Pete Moore. Inside Science No.66. *New Scientist*, 13 Nov. 1993.

²CAM Michaelmas term 1993 p.30

³Organization for Economic Cooperation and Development, Bull & Holt.

⁴Bull & Holt, 1982

⁵Leaflets issued by ABA, PO Box 303, Clayton VIC 3168, Australia, 1990.

⁶Chalak, David A. (1990) "Genetic technology transfer: is the USSR ready?" in Licker, Paul S. ed., *Technology Transfer: Global, National, Corporate*. Calgary: University of Calgary, Faculty of Management, 25-41.

⁷Interview with Dr. Robert Church, University of Calgary, *Globe and Mail* Report on Business 1987 12 09, B26.

⁸Durham, Michael, "Scrambled gene cuisine for dinner." *Manchester Guardian Weekly* 20 Oct. 1996, p.24.

⁹*Environment Views* Spring 1994 p.18

¹⁰Dan Westell "Canola genes altered for profit." *G&M* 5 Apr. 1995, A1, A6.

¹¹Brewster Kneen, The Rape of Canola (reported by Dan Westell, *G&M*, 5 Apr.1995, p.A6.)

¹²Bailey, Diane "A pharmaceutical garden of Eden." *Globe and Mail*, 27 January 1996, D8.

¹³Dr. Lawrence E. Bryan, *Catalyst*, University of Calgary, March 25, 1987, p4.

¹⁴Obituary, George Köhler (MGW April 1995)

¹⁵*Financial Times of Canada*, August 21 1993.

¹⁶Australian Biotechnology Association leaflet No.5 -1990.

¹⁷This sentence is taken from a pamphlet circulated by the Campaign to Ban Genetically Engineered Foods. Its veracity has not been confirmed.

¹⁸Bull (1982, p.31)

¹⁹Ref. 6 in WP doc. *New Scientist*, no. 1636, p.36

²⁰*Environment Views* Spring 1994 p.19

²¹Attributed to a team of eminent scientists led by Dr.Daniel Rudman, Medical College of Wisconsin. (Report by Bill Lawren In *Calgary Herald* April 11 1991, E14)

²²This was done in Monterey, California, without EPA approval. David Baltimore attempted to justify the action in an article "Setting the record straight on biotechnology" (*Technology Review*, Apr. 9, 1987, p.38-46). Dr. Margot O'Toole claimed that Baltimore's own work had been fudged. She lost her career over the exposure ("What price justice?" by Dan Greenberg. *New Scientist*, 4 Mar 1989, p.65).

²³Department of Anthropology, University of Calgary.

²⁴Rifkin, J. "Perils of genetic engineering" (*Resurgence*, March/April, 1985, p.4-7)

²⁵"Lyn Margulis: Science's Unruly Earth Mother" *Science*, v.252 (19 April 1991) p.378.

²⁶*Manchester Guardian Weekly* 15 June 1986)

²⁷Bouma and Lenski (1988), *Nature*, v.335, p.351: *New Scientist*, 29 Sep. 1988, p.42.

²⁸Bailey, Diane "A pharmaceutical garden of Eden." *Globe and Mail*, 27 January 1996, D8.

²⁹Lowey, Mark "Altered food labelled dangerous." *Calgary Herald*, 19 November 1996.

³⁰*Trends in Biotechnology* v.12, 346-352. (Reference not checked, as document missing from library on 1996 11 21)