The Genetic Revolution

Here follows an account of what used to be an extremely long and complicated job: altering genetic code.How do you produce a new species to order?Are there any limits to the options open to bio-designers?

Patents for invented species

You can see how important creating new species is becoming by the intense legal debate over whether or not a new species can be patented.This is driven by huge commercial interests.A "master" patent on a genetically engineered protein has twice been overturned by British courts recently (150).The European community seems likely to pass a law treating animals and plants as genetic inventions, protected under patent (160).Meanwhile US congress has been debating whether or not human beings can be patented as inventions in a similar way (170).Genetic research "will produce beings who fall halfway between what we currently think of as "animal" and "human".It is unclear on which side of the legal line these creatures will fall" (170).In April 1988 US Congress eventually decided that human beings could not for the moment be patented.However there was no clear defini -tion of what exactly is a human being.After all, how much genetic code do you have to change?It is all very well to see you cannot patent a whole human, but as we have seen, the only thing worth patenting is the contents of a single human nucleus containing altered DNA.What about human cells altered and growing in a laboratory flask?If these genes were transplanted into an egg a new human strain could emerge.Therefore I suspect that by patenting genetic code in single human cells it

may be possible effectively to patent a human being legally already.

This is a vitally important area requiring a global legal framework in our global village.It also requires those writing legislation and voting on it to be fully informed.This problem of definitions is going to make effective laws increasingly difficult to write.

Although such arguments sound absurd, without such legal protection companies are unwilling to invest heavily.After all, the effect could be identical to robbing a computer software company by copying a floppy disc containing an expensive computer program.All you have to do is get hold of one altered plant or animal and you can clone away new perfect copies indefinitely (180, 190).

Such laws are raising immense ethical concerns both sides of the Atlantic with a group called Patent Concern formed in Europe in early 1991 representing more than 30 environmental, animal, welfare and religious groups including Greenpeace, the RSPCA, the Genetics Forum and Christian Aid (200).

In June 1991 the Nuffield Foundation in the UK set up a new Council on Bioethics under Sir Patrick Nairne to address moral and ethical issues.However as we will see, all this activity comes rather late in the day, following a long way behind what scientists are actually doing.We will look more closely at the whole issue of law and regulations in a later chapter (210).

Once laws have begun operating in various countries (220) giving companies the right to own different species of plants or animals we have then also begun to see massive legal disputes between companies trying to prove who first created the new genetic code for a new plant or animal (230).However patent protection is one thing, having the licence to manufacture or to sell is quite another with some countries proposing or implementing bans or delaying permission pending investigations (240), even where patents have been formally registered.This whole area is becoming increasingly political (245).

A genetic wordprocessor

Assuming for the moment we lay aside all ethical and legal debates, there are several approaches we can take to designing life:they all work by treating genetic code as a language text on a computer in exactly the same way that this book was programmed onto a word-processor.

With a word processor I can alter this book in various ways:

I can delete text and retype just the bits I want to change. I can of course wipe out the whole thing and retype it from scratch.There is another interesting feature of the word processor which is to borrow sections of text from elsewhere; to insert the text from a previously published magazine article into a certain chapter, and then to trim out the bits I no longer need.

All these same techniques can be used by the genetic writer: writing from scratch works all right if the piece is short and you know what you are doing.Deleting or inserting a minor change is also possible.Inserting a large chunk of genetic writing from elsewhere (another organism) also works well, and is the simplest thing to do.After all, at least you know the code inserted has some biological effects in at least one species.

However, the genetic writer still suffers from a massive disadvantage.The only parallel I can give is a magazine editor who speaks only a few words of Arabic having to assemble and edit a weekly Arabic magazine.On his table he has around 150 pieces of writing of varying lengths and an English two sentence summary of the rough contents of each piece.

He has no-one to help him except a proof reader who tells him if it makes sense or not, but not what the errors are or how to correct them.He has a dictionary of less than 30 words - nothing else.

There is one very time-consuming way out of this mess.The editor can take steps to identify the most important sentences in each section of text by cutting the text up into many different pieces, preferably with obvious markers such as pictures still attached and try them out - asking what each story now contains. By this method he may eventually learn to recognize key words and phrases and what they mean.More importantly he may be able to assemble the magazine very quickly because he links in his mind particular pictures or illustrations with various key items so he does not have to translate them each time.

Swapping new genes for old

The genetic editor uses a range of similar techniques to swap new genes for old ones (250).Let's take for example the problem of the diabetic.Insulin is needed by the body to use sugars properly.In those with diabetes, the Langerhans cells in the pancreas no longer produce sufficient insulin.Without insulin sugar is absorbed into the blood from food but does not cross cell walls so people lack energy, cannot think clearly and can even die.Insulin can be extracted form the pancreas of cows or pigs but it is impure and the body reacts to it.Nevertheless, such extracts have successfully treated diabetics for many years.

Can we find the genetic code for insulin?Can we then programme new cells to make pure human insulin?The first job is to find the genetic writing for insulin.This is like looking for a needle in a massive haystack.

One thing we can try is to cut up the entire genetic code into thousands of pieces of varying lengths and insert pieces into bacteria to see what happens.

To do this we need special biological machines called enzymes.Enzymes are what digest dirt in many washing powders.Enzymes either split chemical structures into two or join them together.We can only analyze genetic code if we have a very large number of copies of the piece to be read.Therefore we need a reliable duplicating machine.This can either be achieved in cells or externally.Rapid progress now means that this complicated process can be completely automated in the test tube (260).

Once the fragment of genetic code has been copied sufficiently and then separated we need to find a vehicle to carry one piece at a time into bacteria.Bacteria are simple single-celled organisms that live, breathe, sometimes move, and produce not only gases such as carbon dioxide but often poisonous substances as well.The type of bacteria usually used in experiments is called E.coli, a relatively harmless germ that lives in the gut and helps us digest our food.

Viruses to reprogramme cells

Bacteria like humans get all kinds of viral infections.Bacterial viruses are called plasmids and work by transferring new genetic code from one bacteria into another (270).Human viruses do the same.The AIDS virus, HIV, for example inserts new genetic code into the soldier cells (T4 white cells) that your body uses to fight infection.When the genetic code is added to the chromosomes in the nucleus the soldier cell effectively loses the instruction sheets on fighting infection and gains instruction sheets on making new virus particles.

Plasmids have been studied a lot in the laboratory (280).We know a lot about them because bacteria pass pieces of genetic messages to each other all the time in the human gut using plasmids.

If a few bacteria become resistant to a new antibiotic, the genetic secret of how they manage it quickly travels to other gut bacteria so the other types of bacteria also quite literally learn new ways of avoiding damage from the antibiotic (290).This is a very important reason why many doctors now try to avoid using antibiotics unless they really need to - we do not want to land up educating a load of plasmids!

Plasmids do in fact exist very widely in the environment and their effects are seen particularly in places where bacteria are adapting to new habitats or where their environment is changing.The viruses are particularly seen in excreta from man and animals where antibiotics have been used, where antibiotics find their way into sewage (often excreted unchanged in urine or as a result - say - of disposing of unwanted medicines down the drain), or from industrial contamination.Industrial discharges containing toxic heavy metals will also induce plasmid led adaptations (295).

Incidentally, industrial wastes produced as a side-product of the chemical industry are becoming more and more of a problem to dispose of safely.Increasingly the industry is looking to genetic engineers to produce bacteria to eat these toxic substances (296), breaking them down into non-toxic residues (297).

One example recently has been the problem of what to do with tens of thousands of East German cars called Trabants.These were produced prior to the collapse of the Eastern bloc with the opening of travel restrictions to the West.Much loved by some and hated by others, these primitive two cylinder cars were are highly wasteful of petrol and fill the air with higher

than normal concentrations of polluting gases.

However the biggest problem of all is presented by what they are made of.Unlike most cars in the West, the bodies of Trabants are made of a synthetic resin which does not rot or rust and cannot be burned because burning releases highly toxic gases.A recent newspaper report claimed that genetic engineers were working on a new type of microbe to eat these vehicles, turning the bodies into a harmless sludge.These changes will also be made using plasmids.

Returning to the problems of plasmids being released or multiply -ing in the environment, we find that prior to the antibiotic revolution in farming and medicine, plasmids were relatively uncommon.Now their distribution as bacterial viruses is vast in both terrestrial and aquatic environments (300).

Recent research off the coast of California has shown these viruses are now multiplying in their tens of billions to form such concentrations that even in seawater, their density is enough to transfer data from one bacterium to another (305).

It is a relatively simple matter to place pieces of genetic writings into plasmids.Enormous advances have been made in the last two to three years (310).Plasmids can then be mixed with E. coli bacteria or with other bacteria.Very occasionally the results can be spectacular although this is very much a hit and miss approach.

Bacteria can be separated easily by taking a metal probe and dipping it into a solution containing bacteria.The probe is then scraped in a zigzag pattern across a small dish containing a special jelly called agar.The dish is then placed in an incubator at blood temperature for several days.Towards the end of the zigzag pattern the number of bacteria still left on the probe was so low that only a very few bacteria landed up on that part of the jelly.Each will now have multiplied rapidly to form a small sticky mound, a few millimetres in diameter.Each of these mounds is an individual colony from an individual bacter -ium.

If we had added a special marker - such as a piece of code for antibiotic resistance - to the piece we are looking to test then we will immediately be able to spot the one in a hundred which have been successfully reprogrammed because those will be the only colonies that tend to grow in agar mixed with antibiotic.

These colonies can then be tested for any unusual properties which will tell us what the piece of genetic code inserted is designed to do.

Artificial Insulin from genes

After enough attempts you may discover an extraordinary event taking place:one of the reprogrammed bacteria in your test tube may have learned to make a human substance - maybe even something useful like insulin.

Such an event only has to happen once in a long time to keep the scientists happy for years.After all, this reprogrammed bacterium can now be cultured separately.Each time it divides it produces another insulin-producing organism.By filling a big brewery-style vat full of warm liquid and food we can start off a process as large as brewing beer except that in this case we are brewing insulin.It will need careful extraction and purifying from other parts of the brew using genetically engineered monoclonal antibodies (p), to remove any dangerous substances from the mixture.

By the late 1980's bacteria were already being used routinely to produce the first genetically engineered insulin.This insulin has now almost entirely replaced cow and pig insulin (320). Man y other remarkable successes have followed for example producing vast quantities of fragments of Hepatitis B virus in a big fermentation vat, using a yeast called saccharomyces (330).These particles are harmless and can be injected as a vaccine for Hepatitis B.Industrial scale production of genetically engineered products is now commonplace (340).

Other ways to reprogramme cells

Scientists have now perfected a somewhat different method for changing genetic code in mammal cells called eletroporation.This uses a high voltage electrical discharge to make cell walls "leaky" so that genetic code (DNA) in the surrounding liquid can find its way into the cell (350).Around one in a hundred cells can be "transfected" in this way (360).This has the advantage over a number of other methods of reconstructing animal and plant cells - these include microsurgery, the use of polyethylenglycol with a virus type called sendai (370), or a

technique known as erythrocyte ghost fusion (380).Reprogrammed mammal cells can either be used like bacteria, growing them in a flask in a factory, or they can be transplanted back, turning the whole animal into a factory production unit.This has been tried in mice, reprogramming skin cells to produce Factor 9, needed to treat a blood disorder related to haemophilia (390).Bacteria are only suitable for producing relatively simple substances.More complicated proteins require the extra machinery in mammal cells (400).

These experiments have basically worked through the "cut and paste" principle: cutting up a piece of text with a pair of scissors, shoving it into a different book altogether and seeing how it reads.Similar progress has also been made on reprogramming yeasts (or fungi) (410).

With bacteria, the results are usually obvious fairly quickly but when genetically altering plants and or animals, it can take months to tell if you have been successful or not.The plant or tree has to grow to produce a crop or fruit you can test for example.However, there are ways of shortening the whole process.If you can find a marker, as we have seen in the earlier example, you are more than half way there.

How to detect success

Markers are some pieces of writing that are on the same strip of code or very close to it, that produce an immediate result.For example, suppose the genetic code from one type of rosebush with ugly flowers programmes it to produce a natural substance which kills greenfly.Suppose also that you notice that a nearby part of the code also tends to turn new rose shoots bright yellow.

When the experiments are complete, a quick inspection of the greenhouse can show you the plants with yellow shoots which have almost certainly taken all the new code, also producing greenfly --resistant roses.Another example currently being used is a gene which gives human cancer cells multidrug resistance to therapy.This is easy to spot if it is taken up by cells in the labora -tory.If joined to a less immediately obvious gene we can tell rapidly if reprogramming with the second gene has been successful (420).We do this by exposing cells we hope have been reprogrammed to the toxic chemicals.Those that survive are worth looking at further.

There is another possibility:how about actually learning to speak the language of the

nucleus?It would greatly assist the editor and would mean he would be able to write genetic code of his own instead of always editing text from elsewhere.

We have learned a few words and phrases: for example we now know of course the exact sequence that programmes for insulin (430) and that for Factor 8 needed by those with haemophilia.The Factor 8 code, analyzed in 1984 was an extraordinary feat since this massive gene was a full 0.1 percent of the total X chromo -some (440).We have also now identified and analyzed the giant Duchenne muscular dystrophy gene which causes muscle wasting (450).We have also recently identified the gene that causes the most common type of inherited mental handicap: the fragile X syndrome.This affects one in a thousand children, almost all boys, and causes mental retardation (455).

Writing out all human genetic code

But now a much more ambitious task is under-way.This task is one of the most daunting scientific challenges ever attempted.The task is to write out the entire human genetic code, letter by letter from start to finish.The code as a whole is known as the human genome and as we have seen it is millions of characters long.Many hundreds of research scientists in a number of countries are racing against time to crack this code of human life.When it is done they think they will be able to begin to work out a vast dictionary showing the meaning in terms of function of each small word or sentence.The process is likely to take less than fifteen more years.It is an expensive business however.The Cystic Fibrosis Foundation alone spent ?75 million on the Human Genome Project in the UK between 1985 and 1989 (460).As a direct result we now know the exact sequence of code for the fibrosis gene, after processing and analyzing almost 300,000 letters of genetic writing.The discovery has huge implications for diagnosis and treatment (462).

This vast project only sequenced less than 0.01 percent of the total human genome of three billion characters.Therefore the whole genome will cost around ?650,000,000 to translate.Who will own the information (470)?Such progress raises urgent ethical questions which we will look at later (475).

Reading genetic code is immensely complicated - or rather it used to be.Sequencing work done by hand used to take months - just to decipher several thousand letters of code.Now a similar process takes less than a week and is almost entirely automatic -thanks to the computer technology of the previous decade.

This whole field is known as microchemical instrumentation (480), and similar machines can now write code as well as read it.

Effectively you can type in a sequence up to 50 characters long, press a button and come back in a few hours.The genetic code will be assembled and waiting for you.The machine is only the size of a desktop computer, and is available by mail order advertised in many scientific magazines.The process used to be very laborious and experimental (490).

Kits to join genetic code strips together can also be bought cheaply, together with genetic code duplicators.These kits are not much larger in size than five copies of this book laid on top of each other.You can see these items on show at the Science Museum in London and even decode part of a gene yourself using a computer simulation (492).A new computer programme is also available now to help design new plasmids and to facilitate in cell cloning operations.The programme is called Clone 3 (493). New methods are being developed continually to speed up and simplify the process from gene sequencing to reprogramming to the end result of protein production (494).

One of the quickest ways to duplicate genetic code is known as the polymerase chain reaction (495).This has revolutionised DNA technology as it allows virtually any nucleic acid sequence (DNA) to be generated in the test-tube in large amounts.The DNA produced is pure and the procedure is much faster than using cells to reproduce it.It is also about to become an important diagnostic tool in microbiology.Practically even a single bacterium, virus particle or parasite can be detected by it (495), and it can also be used in forensic medicine to analyse samples or in archaeology to analyse plant or animal remains (495).

A complete directory of all genes located in humans is now in its ninth edition and has 5,300 entries of which 2,000 genes have been mapped to specific sites on chromosomes (500).

The implications will be beyond measure:if you consider the genetic code as a massive long line of on/off switches with labels, within the next few years we should be able to engineer small changes precisely where we want and know where the result will be.In the US the National Institute of Health (NIH) has set aside ?150 million for the Human Genome Project over the next two years, headed up by James Watson, co-discoverer of DNA structure in 1953.He thinks it will take 15 years at a cost of ?2,000,000,000 (510).

The Director of Research at the Imperial Cancer Research Fund recently estimated that if the cost were spread over 15 years, split between Europe, the Americas, Japan and Asia would give Europe a bill of ?30 million a year of which the UK might need to contribute ?11 million a year (520).The Human Genome Organisation has 250 members from all over the world.

Some very odd questions

It allows us to ask some very interesting questions about patterns of life: questions that may at first sight appear bizarre but which are of fundamental importance to us in designing new species.Can farmers produce better animals for eating (525)?Do elephants have to be so large - can't we programme miniature ones?Can we programme into chimpanzees a portion of human code that gives them a limited spoken language capability and better reasoning powers?Can we use them as intelligent sub-human clones for difficult and dangerous tasks instead of incredibly expensive and limited robots.Before we know where we are we are back into debating what constitutes a human being: how much human code do you need - 5%, 10%, 55%?Is it just appearance?How human do you have to look?The issues do not just relate to patents but more importantly, to ethical licensing.

To even ask the questions is to risk arousing the most intense controversy (525).

By comparing the genomes of different people (530) and different animals it should be possible to build up a vast vocabulary accurately.The interesting thing is the unity of the genetic code: a yeast cell uses exactly the same coding language as a human.Therefore the same technology works for reprogramming cells from humans or other mammals (535).

One of the difficulties in reading the code and knowing what each piece does is that large amounts of code are only used in the developing embryo.For example, the instructions to help form the eye will only be triggered in some cells in an embryo at one critical point in development.For the rest of the entire period from egg fertilization to death of the animal, that strip of code is locked away, turned off or inactive.The secrets of these areas of code are perhaps the most fascinating parts of life itself.

Why does the human hand produce five fingers and not six?Which set of genes tells fingers to produce nails on the top and not along the bottom?Could a human have four sets of arms and two pairs of hands?Occasionally drugs given during pregnancy produce such events - usually because of confusing cells as to where they are rather than because of direct genetic damage.

Embryo experiments

Scientists are busy altering genetic code in fertilized eggs and watching to see what happens to the embryos.In drosophila insects the genes have been identified for early body formation, and adult skin production (540).We can expect similar progress in mice and monkeys.It will be very tempting for some to try the same with surplus fertilized human eggs resulting from fertility treatments such as GIFT.Many of these techniques involve giving a special drug to a women, stimulating her ovaries to produce up to twenty eggs instead of only one.These are then removed in a minor operation.

In many centres, all are then exposed to sperm and observed under the microscope to see if they are dividing (i.e. successfully fertilised).Some eggs (two to six) are then implanted in the womb in the hope that at least one will implant successfully.Quite often none do although sometimes several succeed, and the result is triplets or quadruplets or quintuplets or sextuplets.The big ethical question is what to do with the spare embryos, which can be frozen indefinitely.Many are being used in experiments, most of which involve allowing them to grow and develop in the laboratory.

At this point you may like to pause for yourself to consider some of the more bizarre possibilities: an animal with the flesh of a cow, the milk of a human mother, the wool of a lamb, the tolerant digestive system of a pig (550).You can design one of your own (560).

This may all seem rather far fetched to you.Even as long ago as 1987 (light years ago in bioengineering progress) it was reported that artificial chromosomes were being manufactured for yeast cells with the prediction that production of entire chromosomes for more complex organisms would be possible in the near future (570).

I leave the final word here to a well known writer - "If human blood cells can grow outside the human body, why not human bone cells, muscle cells and nerve cells?And eventually all of

them together functioning as a single living organism" - words written long ago in her novel "Frankenstein: a modern Protheus" by Mary Shelley.However the thought has recurred (550).

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