The Genetic Revolution

From the most ancient times a rule of life has been seen to be true: insects breed insects, birds breed birds, cows breed cows and humans breed humans. If you take acorns from an oak tree and plant them the result is more oak trees. Creatures and plants remain true to type, faithfully passing on their characteristics from generation to generation. Where thereare slight variations, for example in skin pigmentation or in the colouring of flowers, then these too mcan usually be traced down the generations. The basis of life has been remarkably stable considering its complexity.

The basis of this inheritance was not understood however. An understanding of how organisms are built out of cells only emerged with the invention of the first light microscope by Robert Boyle in the eighteenth century (check date). It was many decades later before we began to understand how the cell works. Most of the structures in a cell could only be seen with the high power of the electron microscope.

However, for many centuries experiments were already taking place with cross-breeding - the earliest technique of genetic engineer -ing.

An Austrian monk

In order to understand the mechanism of inheritance, we need to start in an Austrian monastery around 1760, in the potting sheds of a gardener called George Mendel.This monk was curious to know what would happen if he took pollen from one type of plant and used it to fertilise another.Would the pollen be accepted?Would it succeed in fertilising the plant?If it did, would seed result which would germinate?Finally, when it germinated, what kind of plant would grow?

For thousands of years previously such attempts had been made with animals.For instance, in the time of Jesus, it was common to allow a horse to mate with a donkey:the result was a cross-fertilised egg which went on to develop into a rather strange-looking creature at birth.The creature had some of the best characteristics of both parents and was known as a mule. This new species had one important drawback: you could not breed from it because it was always sterile.

Hundreds of others examples could be given over previous centuries of selective breeding - indeed Jacob in the Old Testament seemed to know what he was doing in selectively breeding white and black sheep to produce a herd entirely coloured as he wanted, at a time when sheep ownership was being determinedsolely by colouring of their woolen coats.

The process of inheritance has been well understood by families who observe - say -grandpa's orange hair through to a grand -child or other family likenesses.However, the mechanism has only relatively recently been fully understood.Why was it that dark haired parents could occasionally produce a fair-haired child ?

Cross-fertilisation

Mendel was interested in all this. Moreoverd the monastery stood to gain from improved strains of cereal plants. Mendel found that when he cross-fertilised closely related plants with obvious differences, he got neither a mix nor equal numbers of aech type. Instead he found a curious pattern. After a while he found he could predict in advance not only what variations he would see, but also how many of them.He realised that in each seed there was a lot more information stored than would ever be used to form the new plant.

Some of this information it seemed was hidden away in many plants and only expressed when

cross-fertilisation took place. It seemed like each plant had its own strong and weak features. Weak features only came to the surface under certain circumstances. These strong features have become known as "dominant" while those which tend to be hidden away are called "recessive".

This same information and understanding is used daily in dozens of Genetic engineering laboratories all over the world every day. When he cross-ferti -lised tall and short varieties of the same plants he found he always landed up with seeds that produced plants in a fixed ratio of three tall to one short (check which way round). From this he prposed a theory which was to revolutionise our thinking about breeding.

He came to the conclusion that each plant must have two sets of instruc -tions for each part of its structure. Therefore each plant had two set of instructions for height. However if the plant had a mixture, then the tall one was always dominant.

You can see how this works in Fig 1. When sperms or eggs are made - or their equivalent in plants - the original cells divide into two, with only half the full set of instructions needed for life in each half. So parents with a mixture of tall and short instructions in their cells will produce sperm or eggs with either one or the other.

Fertilisation happens when pollen and ova meet, (or sperm and eggs in animals). When this happens, the new composite cell ahs a complete set of instructions and is able to start forming a new plant. Clearly four types of plants could result: one type where both pollen and ova have provided tall instructions, another where both are short, and two where there is a mix. Three of these out of four will be taall. The only plant type that will turn out short will be the one where both sets of instructions are short, because both parent plants passed on the recessive gene.

Fig 1

"Mother" "Father"

T S T S

Both these plants have a tall gene in the pair so both are tall.

Fig 2

1. Mother Father

 T T A tall plant

2. Mother Father

 T S A tall plant

3. Mother Father

 S T A tall plant

4. Mother Father

 S S A short plant

So in his classic experiment:two tall plants cross-fertilised produced short plants one time in four.Interestingly,if short plants are only fertilised by other short plants then you can see that no more tall plants will ever be produced.A new strain will have been created.

Simple methods like this have been widely used by gardeners and horticul -turists for over a hundred years: selective breeding from plants showing the characteristics you want to encourage. The development of pedigree dogs is an ancient art which has worked on the same principle: only allowing dogs to mate that have the right characteristics.

Incidentally you can see straight away a major problem: if you go on inter-breeding from just one small group, then more and more recessive genes may emerge. Some may have hidden dangers for the animal. Take dogs again as an example: in the wild they breed widely producing a group of fairly even appearance. If bad traits emerge , they tend to be eliminated because they dogs do not survive long enough to breed or because the recessive traits are covered up by dominant genes from others in the group. However in domestic breeding, the dominant genes are being deliberately trimmed out. The result is a beautiful breed but one which may be susceptible to a high rate of blindness, tumours or hip problems for example. Ther are many inherited disorders in humans that can arise in a similar way.

Haemophilia and human inheritance

Take haemophilia for example:haemophilia is a disease where blood does not clot properly so people can in severe cases bleed to death. In the last century haemophilia was known as the Royal Disease because it was so common in the Royal Family. The reason for this was of selective breeding. The gene causing the problem is recessive and emerged when members of the European Royal Families continually inter-married.

The result was a "pedigree" with the same kind of problems as in over-bred animals. There is then a biological basis for the Biblical injunction against close relatives marrying. Cross-fert -ilisation is needed to keep us all healthy.

In those with haemophilia the substance which is missing is called Factor 8 - a substance which is found in normal blood and which is one component of the clotting mechanism.Factor 8 can be extracted from blood donated for blood transfusion, although the process is complicated and expensive. If someone with haemophilia is bleeding uncontrollably from a cut an injection of Factor 8 stops it very well.

The Extraction process has turned out to be very unsafe however: all over the world, the virus causing AIDS (called HIV) found it's way into donated blood. Whereas an infected blood transfu -sion to an uninfected person only results in one new infection, Factor 8 is obtained by pooling plasma from a very large number of people.

Just one donation in a hundred can be enough to contaminate the whole process so that dozens become infected from the injectedFactor 8. One reason why Factor 8 supplies before 1985 were so dangerous is that the UK depended on Factor 8 imported from the US. In that country blood donors are paid with the result that many drug addicts donate blood to raise extra income. This greatly increased the risk of hepatitis B virus or HIV finding their way in to the blood banks. Effective testing from 1985 (using techniques derived from Genetic engineering) has almost eliminated this risk. However, by 1985 in the UK over 100 men and 250 boys were already HIV infected through these treatments.

In addition to blood testing, special treatments since 1985 have made Factor 8 particularly safe. Nevertheless the pressure has been growing to make Factor 8 in the laboratory. In 1984 the genes programming for Favtor 8 were identified for the first time, copied, and analysed (10). In the last two to three years Genetic engineering has now been used to programmecells from mammals grown in the laboratory or in the factory to produce human factor 8 (20). We will be looking at this remarkable achievement in more detail later on.

Why are all those with haemophilia men? Mendel's experiments explain to us why:most people have a pair of genes to tell cells how to make Factor 8.Even if one gene is faulty or missing, the process can continue.If, however, as a result of an unlikely and unhappy accident, a man and woman who both have a faulty gene have a family then Mendel would tell us that on average one in four of their children will inherit two faulty genes and be unable to produce Factor 8.Two others will be carriers and the fourth will have both genes intact.

The interesting thing about haemophilia is that the gene carrying information on blood clotting just happens to be sitting on the X or female chromosome.This "linkage" of one characteristic (sex) with another (clotting) is extremely important to the geneticengineer as we shall see later on.Linkage with an outward obvious sign is a good marker of other genes also inherited in the "package".

Genes and Chromosomes

All the genes or sets of instructions in a human are contained in just 46 chromosomes, each of which exists as one of 23 pairs. Chromosomes can be seen under the microscope and most look quite similar.However, there is an obvious difference between men and women:women have a pair of chromosomes shaped like two Xs while men have one X chromosome and another shaped like a Y.The Y is dominant so a single Y produces a boy and suppresses a single X.The haemophilia defect is on the X chromosome so women never get the disease although they can be carriers, with their second normal X chromosome dominating over the abnormal one.

Genes and Cell Factories

So now we understand what genes are and how they are inherited we can begin to see where scientists can start to make changes and how scientists can make artificial chromosomes (strings of genes) (30).There is one further thing we need to understand.While every human cell (except red blood cells and sperm or eggs) contains a full set of chromosomes with all the genes for the whole person, each cell only uses a minute fraction of the information.

One of the greatest puzzles in medicine is how a kidney cell knows it is a kidney cell and not a piece of skin for example.The chromosomes are the same.The genes are the same.The full genetic code is the same. We need first to understand how a cell works: each cell in the body has a similar structure. Incidentally you will find an almost identical structure in the cells of every living creature.Cells are tiny.Around a million will just about cover a square measuring one centimetre by one centimetre. Each of these cells is basically a chemical factory with three parts; a brain (nucleus), cell fluid (cytoplasm) and a cell wall to keep it all together.

1. Cell Wall

All cells are like tiny balloons or bags.The bag itself is made of a special membrane which functions like the wall of a fortified city: it keeps things in and others out.There are gates in the wall which open or close at various times to take in food or dispose of manufactured goods.

There are also pumps in the wall which push substances in or out. These pumps are like air

conditioning units in an office block. They keep the internal environment constant, whatever is happening outside.The water inside may need to be kept saltier for example, or there may even be a need for there to be an electrical charge stored inside the cell like a tiny battery. This means that when the cell wall gates are suddenly opened, a current can flow - this is how nerve cells conduct electrical impulses.

If cell walls are exposed to various chemicals, they become leaky, not only allowing unusual substances out but also allowing all kinds of things to drift inside the cell from the surrounding fluid.This is very important for the genetic engineer.A favourite trick is to place cells in a liquid containing fragments of genetic code, and make the cells leaky so they can move inside.

2. Nucleus

Inside the cell there is a bag within a bag.This second bag is much smaller but has a similar function.This bag keeps all the chromosomes together inside the cell.Every instruction the cell needs is in the nucleus.The nucleus is the equivalent of the cell brain, or the controller of a factory.

3. Cytoplasm

Outside the nucleus, the rest of the cell is far from empty: the space is stuffed full of a maze of tubing, called endoplasmic reticulum, as well as factory assembly units called ribosomes, and power supply units called mitochondria.

Since chromosomes and their genes never leave the cell nucleus, how do the ribosome units know what to make and when?There is a special communica -tion system which takes messages from the inside of the nucleus to the ribosomes assembly lines.It works on the same principle as a fax machine or a photocopier and courier service.These very same principles are used by genetic engineers all the time to copy instructions.But first we need to understand a little more about how a chromosome stores it's vital information.

How chromosomes store information

If you take a chromosome apart into its tens of thousands of genes and take each gene apart one by one, you will find each one is made up of a long string of building blocks or molecules. There are around 3000,000,000 building blocks used in every human cell (35).These special strings of them are called nucleic acids, because they are chemically slightly acidic and they are used in the nucleus of a cell.The nucleic acids themselves are called DNA (or deoxyribonucleic acid).DNA is built up of only four different building blocks known as bases.

These form a four letter "alphabet" formed from the different shapes of the four structures: Adenine, Thymine, Guanine and Cytosine, or A,T,G and C for short.Assembled DNA consists of two strands that look a little like the model railway track in our home.Each rail is a long string of the four bases in a special language sequence - ATGCCTA for example.These chemicals operate in reverse pairs so that if - say - A is one side of the track, T is always on the other; G always pairs with C and the other way round is also true.The pairs are joined together like the sleepers of a railway track (see Fig 4).

Fig 3: Building blocks or bases forming nucleic acid (DNA)

There is one other curiosity: when the double track is formed, it has a natural coil to it so it circles round and round like a spring.This spring shape is called a double helix (Fig 5). This coiled structure was first discovered by James Watson and Francis Crick who won a Nobel Prize for it in the 1950s.The 3,000,000,000 pairs of basis are held in groups of 100,000 genes or packets of instructions.

Fig 4: The coiling that happens with DNA once made

The language of life

When a gene is dismantled, you can write out the order of bases as a code or language - even with punctuation marks.Typing out the full language from all your own genes would fill more than this whole book.In fact, the lists of instructions are extremely long and detailed - probably enough to fill the entire Encyclopedia Britannica.There is a lot of information which is repeated twice and many pages which are "spare" - filled with a jumble of words and phrases not being

used currently at all.

Each cell in your body contains the full encyclopedia but only uses a few pages.All the other genes are "turned off" or deactivated.To put it another way, the other volumes of the encyclopedia are in the book shelf unopened.

One feature of cancer cells is that the wrong volume of the encyclopedia is open:the wrong genes are active sending "correct" but inappropriate instructions to cells to grow and usually to become less specialised.

Messengers of life

So how exactly are these instructions sent?The cell uses a second form of genetic code called RNA (or ribonucleic acid) to make a precise copy of just one strip of code.The RNA is written also in a four letter code, the only difference being that it uses Uracil or U instead of Thymine or T.Once this messenger RNA has been printed off the DNA using it as a template, it passes through the wall of the nucleus and is carried through the cytoplasm until it finds a ribosome factory. And then the real action begins.

Every structure in the human body is built out of twenty different building blocks called amino acids.Each is the equivalent of as differently shaped piece of lego.The body finds it very difficult to make them which is why we need protein in our diet from meat or plant sources.In our gut proteins are broken down and the amino acids are then absorbed.

There is almost no limit to the shapes that can be built with these amino acids, with a parallel being how many different models can be built with twenty thousand lego bricks of twenty different shapes.The only difference with proteins is that as with DNA they are assembled piece by piece in a long string. However once the string becomes longer, or is completed, the string starts to bend and kink, with curves and straight sections appearing in different places according to which different building blocks are where.

As the folding up continues, building blocks which were in the centre of the long string can

suddenly find that they are almost touching building blocks near the beginning or the end.Some building blocks tend to pull towards each other as if magnetised, and these "magnets" tend to "glue" the structure together and give it stability.If I attempt to draw it for you (Fig 6), it will look just like a coiled piece of string.This is very misleading because the reality is more that of a string of sausage tangled up and pressed down into a soft round ball.

Fig 5: How 20 amino acids in a string form structures

Note: the coiling is seen here only in two dimensions.In real life the coiling also happens towards and away from you in three dimensions.Many such shapes can be seen in the Science Museum in London where they display some of the original models built by scientists to try and work out the structure of proteins.

The ribosome reads the four letter language of the messenger RNA in three letter words.Each of the three letter words is the cells own name for one of the twenty amino acids.The ribosome starts at the beginning of the RNA sequence and reads it triplet by triplet, and as it does so, the factory increases the length of the amino acid chain block by block.As the chain begins to emerge it starts to fold up into it's correct shape (Figs 7 and 8).

Fig 6:The Dictionary of Life

Fig 7: A ribosome factory building a protein

Note: Here the triplet being read is UUU which you will see from the cell dictionary in Fig 7 is the code for an amino acid known by human chemists as phenylalanine.The assembly process is entirely automated.There are 61 different transporters or "fork-lift trucks", each of which exactly fits only one of the 61 combinations of three bases used in the cell dictionary.You will see from Fig 7 that like human language, the cell sometimes has several words that mean the same thing.These are used interchangeably.As soon as the forklift truck latches onto the RNA, an enzyme automatically joins the amino acid to the growing chain and disconnects it from the truck.The process moves along the RNA to the next RNA and repeats until it meets the UAA, UAG or UGA words which are cell language for "stop".

Proteins, fats and sugars to order

Some very complicated structures are formed from several different protein chains - Insulin for example is formed from two, and antibodies which are immensely important to the genetic engineer in medicine as we will see, are formed from four.

Structures formed from sugars and fats cannot be programmed directly by the nucleus as ribosomes can only handle amino acids. To make these other things the nucleus tells the ribosomes to produce particular proteins which are themselves part of a new production line.These special proteins are called enzymes and they repeatedly carry out simple joining up or splitting of identical units in identical ways.

Having looked around inside a living cell and caught a glimpse of the huge range of activities going on, we can begin to understand why it is so attractive an idea to be able to control a cell for ourselves, and take over these amazing ribosome assembly lines for our own purposes.We can also begin to understand how we could do it.After all, all we have to do is get the right message to a ribosome by fixing the nucleus exactly how we want it to work.

Dividing cells - duplicating genes

There is one other thing we must understand before we can go any further in moving from a single cell under our control to a massive chemical production factory using billions of these cells: how do cells divide, and how do they keep their genetic code the same each time?

The process is similar to what happens when messenger RNA is formed from DNA.The coiled structure of the DNA first has to straighten out.Next it is unzipped by an enzyme running from top to bottom.All the natural joins are broken (Fig 9).

Fig 8: How DNA is unzipped in order to allow the cell to divide

Note: As the DNA unzips, loose bases in the liquid of the nucleus get attracted to their opposite numbers and the result is two new double strands, each identical to the one before.

The cell is very vulnerable to interference at this stage and mistakes can happen in the copying process.If they do occur the result is a mutation although the effects may be so slight as to be unnoticeable.Many mutations are lethal to the cell and the cell dies or gets stuck mid-division.

Other mutations may trigger off unwanted effects, for instance damaging that part of the genetic code that enables the cell to understand where it is in the body from interpreting chemicals released by it's neighbours.The result is that cells go on dividing to form benign tumours if the growth is slow and localised, or cancers if the growth is faster with cells breaking of to grow elsewhere.

Cancer chemotherapy hits cells at this vulnerable stage of chromosome duplication, by jamming the dividing mechanism.This can be done by giving someone a slightly altered base as a medicine.The base is used to build new DNA but the process halts when an incorrectly shaped building block is used.

Radiotherapy also attacks dividing cells - this time by firing atomic particles at high speed, from a radioactive source, into a mass of dividing cells.The atomic particles knock out bases from the growing sequence, damaging the cell so it cannot divide properly and dies.The same treatment can of course increase the risks of cancer developing in normal cells, but rather less so.

Reorganising the body

So now we understand how cells work and divide, how is it that cells turn out so differently in the body if all start off with the same genetic code?

Usually, cells in the body become specialised before birth:This process is called differentiation.For example, a nerve cell will always be a nerve cell.In other words, the destiny of each cell is determined in the womb.Cells influence each other by complex signals which are

often using chemicals so that cells landing up in a certain position in a developing embryo are influenced in the way they develop.These chemical signals lock away huge sections of genetic code permanently.So not only does information come out of the nucleus in the form of messenger RNA, but also the part of the genetic code copied in RNA is influenced by messages from the outside of the cell.

It is essential to remember here the difference between "somatic cells" which are fixed for a lifetime in one place or at one job, with a complete set of genes, and "germ" cells (sperm and eggs) which have half of each unzipped chromosome without duplication. Germ cells therefore have 23 half chromosomes and cannot divide until the other halves are provided at fertilisation.

The genetic engineer has two choices:he can alter somatic cells so, for example liver cells in a diabetic start to produce Insulin, or he can alter germ cells so that every cell in the new embryo is reprogrammed.These changes will be passed on from generation to generation for ever or until further reprogramming is done.Laboratories all over the world are already trying out a wide range of such experiments on animals. (40)Many scien -tists accept that with current rates of progress it will only be a matter of time before human germ cells are being reprogrammed routinely (50).This raises huge ethical problems which we will consider along with many others in a later chapter.

However before we go to all the bother of altering genetic code, how about simply copying it to produce an identikit clone or perfect twin.As we will see in a later chapter, this technology is already widely used in animal breeding, so could it be done with humans? **The Genetic Revolution - published 1995 - free book by Patrick Dixon**