

- *Detoxification of poisons*: Many otherwise toxic substances appear to be broken down by the activity of the gut flora.
- *Gut repair*: The leaky gut is of growing interest in health (Chapter 6). Gut bacteria have a significant role in the repair of the leaky gut.
- *Digestion*: By assisting in acid balance and other environmental controls, the gut bacteria aid in the digestion of foodstuffs. They help digest the soluble fibre in food such as beans, lentils and other legumes, which then become the source of the short-chain fatty acids (SCFAs; see below and Chapter 7). Full digestion, of course, is the first step in the reduction of the food reactions that contribute to irritable bowel, migraine, asthma, chronic sinusitis and other related conditions. Gut bacteria play an important role in the prevention of these common health problems.

But there is more. These bugs not only talk to each other (by means of chemical signalling and even the exchange of genetic material), but to the cells of the body. This is proving to be the most intriguing facet of their behaviour, with significant implications for our immune system.

### Gut-associated lymphoid tissue (GALT)

Along the 7.5 metres of gut, and in the massive curtain-like structure (omentum) from which the gut is suspended, lie numerous lymph glands. These glands are in direct communication with the extensive lymph tissue situated in the gut wall. This includes dedicated islands of lymphatic cells (Peyer's patches), and widely disseminated lymphocytes such as T-cells.

Even the appendix, long thought to be a vestigial remnant from an ancestral predecessor, is now considered to be a significant part of the GALT. It is interesting to note that Crohn's disease sometimes first shows up in the appendix. As Crohn's is an immune disorder, its presence in the appendix may signal an immune system under siege.

The immune system of the gut is separate from the immune system of the circulation, and it functions differently. The lymphocytes of the gut 'home' back to the gastrointestinal tract, and do not remain in the mainstream circulation. This is in direct contrast to the rest of the body, where lymphocytes readily transit from the lymphatic circulation to liver, from liver to bloodstream, and back again.

This does not imply that these two arms of the immune system do not talk to each other. They most certainly can and do, through the cytokines and other immune messengers. The real point here is to understand just how specialised and important the gut and GALT are.

Foreign matter can enter our bodies through the air we breathe, through the skin, through sexual contact, and through the gut. Under normal circumstances, the amount of germs, pollution, pollen or dust-mites we inhale would be measured in micrograms to grams. The skin is such an effective barrier that it takes a significant wound to bring more than a tiny amount of anything in contact with the body's tissues. (The subject of deliberately injecting foreign matter was dealt with in Chapter 2: Vaccination; but this, too, is usually in very small amounts.) Seminal fluid is measured in grams.

By startling contrast, the amount of foreign matter, including highly reactive foreign proteins, which goes daily through the gut in the form of food and fluids is measured in kilograms. Most of this is not just in transit. It requires active absorption and assimilation, and may be 'contaminated' with immeasurable numbers of micro-organisms. It is hardly surprising therefore that the gut should have a sophisticated immune system of its own.

This is perhaps best imagined if you think of drinking a glass of milk. Assuming you are one of those who still produce the gut enzyme lactase into adulthood, and that you have no antibodies to milk proteins, then within an hour or so you will have digested the milk. Proteins, fats and sugars in the milk will have entered your bloodstream, providing you with amino acids for tissue regeneration, and fats and sugars for energy.

If by contrast, we draw that same glass of milk into a large syringe and inject it slowly into your vein, within an hour or so you will be dead, or in the intensive care ward fighting for your life.

What has gone on in the gut that renders this glass of milk safe for most of us to drink? Why is an injection of milk lethal? Certainly one reason for the danger is the physical properties of the milk. But equally important are the chemical and immunological responses that injecting it would provoke. One factor determining uneventful passage through the gut barrier is the immunological phenomenon known as 'the development of oral tolerance'.

The medical profession has more questions than answers about oral tolerance.

We have learned that it begins at weaning, and that as a result of it, food antigens, recognised as foreign by the immune system of the small intestine, do not cause a reaction because the immune response is suppressed.

We know that breast milk possesses a chemical called *transforming growth factor beta*, a natural immunosuppressive agent. Breast milk may assist, but cannot be essential to, the development of oral tolerance. We know that oral tolerance is essential to survival, and that only minor abnormalities are compatible with life. We know that one of these abnormalities is coeliac disease (Chapters 2 and 6).

Perhaps the study of the gut bacteria is beginning to unlock some of the secrets of oral tolerance. By looking at the bacterial environment in the developing infant gut, maybe we will gain insight into the rising incidence of food allergies and autoimmune disease. For suggestions on maintaining gut health, see Appendix 3.

### Getting it right

If we are to employ our knowledge of gut bacteria to better support our immune system, we need to understand what happens immediately during and after birth.

The general consensus is that *in utero* the foetal gut is completely sterile. During the birth process and the early hours of life the infant acquires and swallows micro-organisms from the birth canal, the hands of those assisting the birth, the skin of its mother, and the nipple it sucks.

Evidence is accumulating that these micro-organisms take their cues from chemical signals along the length of the gut, and, 'as part of an intensively interactive community that has been honed by millions of years of co-evolution ... guides early colonisation'.<sup>7</sup> It seems that the bacteria might actually determine which part of the genome (the collection of human genes in its entirety) in any one intestinal cell might be turned on or off.

It is amazing to think that the actions of the bacteria might have localised effects on cell differentiation. Even more amazingly, the bacteria might also influence the subsequent *behaviour* of those cells. When we pause to consider that this includes immune cells, we realise the immensity of the possibility. The presence of normal healthy gut bacteria in the early weeks of life may exert lifelong immune benefit. Even when the *mother* supplements with good bacteria in pregnancy, the baby's risk of later asthma and eczema is reduced (Chapter 6).

The laboratory evidence indicates that these little bugs are the unsung heroes

of the immune system. Unlike most micro-organisms, many gut bugs can pass through the protective lining of the gut wall and stimulate macrophages (one of the two main kinds of phagocyte). There are studies under way in human babies to determine what effect the bacterial count in early life has on later immune function.

At a clinical level, there is already a wealth of circumstantial evidence. Let us look at some of it.

## Hygiene

Hygiene as a factor in immune disorders is discussed in Chapters 2, 6 and 7. Theories surrounding gut bacteria deserve at least as much attention. The intestine of a three-day-old baby from the slums of Lahore in Pakistan is likely to be teeming with intestinal bacteria, but a Swedish baby may have none, even when it is a week old.<sup>8</sup> This cannot be written off as racial difference, any more than could the differences across the Berlin wall or between urban Aboriginal children in Australia and their desert cousins (Chapter 6).

Birth-order also has a significant impact on the later development of allergic illness. Firstborns have more allergic problems than their siblings. And the benefits seem to accrue as the number of children in the family increases. There are a couple of possible explanations for this. One is that older children bring home more respiratory germs. Equally important, however, those big brothers and sisters impart gut flora through kissing, sucking the baby's fingers, giving the baby their fingers to suck, sharing food, and so on. 'Wash your hands before you play with the baby' runs counter to thousands of years of natural human behaviour.

Coeliac disease may represent yet another side of this story. In Chapter 2 we saw that its *prevalence* is on the increase, and this increase seems to be more common in developed nations. Asians living in a Western culture move from a low rate of coeliac to a higher one. Research indicates that better diagnostic facilities do not explain these differences. Like most medical problems, the causes of coeliac disease will prove to be multiple. If one in four Westerners have the genes for gluten sensitivity but only one in 25 of those go on to develop full coeliac disease of the gut, what else is happening? We know that breastfeeding is protective, but only partially. Gut bacteria may be the missing link.

## A done deal

What do these little gut bugs ask in return for these multiple services? Their requirements are much the same as our own—congenial housing, shelter, companionship, interesting and productive employment, and a square meal.

For a square meal they like resistant starches and soluble fibre, such as are found in asparagus, sweet potato, onions and garlic, baked beans, red kidney beans, lentils, and the whole range of foods known as ‘phyto-oestrogens’. They also consume some of the sugars, lipids and proteins in the gut, and although this could be seen as competition with our own needs, the losses are small and the benefits considerable. Included in their mixed diet are dead cells from the gut wall, bacterial debris, bile acid wastes, and some surplus cholesterol. In return for a small energy supply, they not only contribute to immune function, they modify blood lipid and cholesterol levels, and act as all-purpose waste disposal units as well.

Matters might end there, with food in exchange for services. But with an efficiency typical of Mother Nature, even the waste products of their digestion prove to be invaluable.

To make beer or wine, we take a sugary liquid and add yeasts. The yeast consumes the sugars, and as a by-product of its digestion gives us alcohol and some vitamins. Similarly, when the friendly bacteria in the gut consume their mixed diet, the by-products are vitamins and some *short-chain fatty acids* (SCFAs). The latter include lactic, acetic, propionic, citric, hippuric, orotic and butyric acid. The SCFAs have various functions that we know about, and others that we have yet to fully understand.

Three SCFAs, acetate, propionate and butyrate, have been shown to affect the manner in which tissues normally regenerate themselves, avoid inflammation, and destroy cancer cells.<sup>9</sup> SCFAs are fundamental to these processes. They readily enter the bloodstream. They are one of the main reasons why gut bacteria can modify, or even prevent, conditions as diverse as irritable and inflammatory bowel disease, various cancers, autoimmune disorders, psoriasis, asthma, eczema and arthritis.

Let us take butyric acid (or butyrate), for example. It supplies up to 70 per cent of the energy required by epithelial cells in the colon. The epithelium is the single layer of cells which lines the entire length of the gut. Butyric acid is also believed to induce enzymes such as *transglutaminase*, which is involved in mucosal repair, and *glutathione transferase*, an important redox enzyme. Various studies have

suggested that a disturbance of butyrate metabolism is the underlying problem in ulcerative colitis. Colonic irrigation with butyric acid has been shown to induce remission in patients with active ulcerative colitis.<sup>10</sup> There is little doubt that butyrate is a key player in the prevention of colonic cancer, and possibly breast, prostate and liver cancers as well. Its role in gene transcription is discussed shortly under Genetics.

SCFAs are thought to be one of the means by which gut bacteria help to reduce the population of undesirable organisms in the gut, including some of the most dangerous pathogens. Acetic acid, produced predominantly by *B. bifidum* and *B. longum* in combination with lactic acid (see below), has been found to inhibit the growth of the organisms that cause typhoid and bacillic dysentery, and the infections caused by salmonella, golden staph and pathogenic *E. coli*.

SCFAs also affect micro-organisms that exist as normal gut dwellers, or 'commensals', but which can cause problems if they increase in numbers or develop into a virulent strain. *Helicobacter pylori*, the much vilified 'causative agent' in peptic ulcer disease, is found in the stomachs of up to 80 per cent of healthy individuals. It is suspected that this organism is problematic only when abnormal conditions allow its numbers to proliferate. In fact, helicobacter is now being researched as 'one of humanity's oldest and closest companions', a possible protective agent against oesophageal cancer and other diseases of the digestive tract.<sup>11</sup> Indeed, there is even research suggesting that infection with helicobacter may protect against obesity (Chapter 8). We know that *L. acidophilus* and *L. casei* control the growth of helicobacter by the production of acetic acid, hydrochloric acid and, particularly, lactic acid. So is the development of peptic ulcers just another example of bacterial imbalance?

Acetic acid, the principal SCFA found in the gut lumen, has been found effective against a wide range of yeasts, moulds and bacteria.

In low numbers, the candida organism is probably a normal gut commensal. Patients who are suffering from a candidal infection complain of feeling 'groggy' all the time. The overgrowth of yeast in the gut can produce alcohol by the process described above. In the presence of sufficient dietary sugar, even the staunchest teetotaller can produce low levels of blood alcohol. No wonder people can find sugary diets addictive, and children living on junk food can behave irrationally. Only in the case of severe immune compromise, such as HIV-related illness or

chemotherapy, are the means truly lacking to bring commensals such as candida under control.

Propionic acid has important roles beyond the gut: it helps balance hormone levels through its ability to stimulate the liver to produce sex hormone binding globulin (Chapter 7).

SCFAs are not the only means by which gut microflora can reduce numbers of unfriendly colonisers. Other by-products of the fermentation process include hydrogen peroxide and carbon dioxide. As already noted, they can produce specific bacteriocins, chemicals which are most accurately designated 'nature's antibiotics.'

Although not part of the gut, the vagina in females and urethra in both sexes are similarly colonised by friendly bacteria. They are both vulnerable to infection, and are normally colonised by specific strains of lactobacilli. Culture of vaginal lactobacilli has found them to act against a wide range of urogenital organisms, including chlamydia, gardnerella, and ureaplasma urealyticum. Any comments in later chapters about the mistreatment of gut flora are pertinent to these organisms as well.

## GENETICS

And the end of all our exploring  
 Will be to arrive where we started  
 And know the place for the first time.  
 – T. S. Eliot, *Four Quartets*

### Chromosomes

It is possible to understand the really exciting stuff about genetics without discussing the terminology or the structure of the famous double helix. This would be a pity, however, as the reality is quite poetic.

The double helix is often likened to a ladder, two longitudinal strands joined by rungs. Because this ladder is twisted, it is best to think of it as a rope ladder. Another image is a double-stranded string of pearls.

One half of this double helix or rope ladder or string of pearls is the basic unit. In chemical terms, the pearls are called *bases*. The pearls are attached to every

second link of a chain. There are two kinds of links and they alternate. In chemical terms, one kind of link is called a *phosphate*, and the other is a sugar molecule called a *ribose*. The pearls are attached to the ribose links.

The two strands lie side by side, with the pearls opposite each other. Each pearl has an invisible bond *across* to the pearl opposite it in the complementary strand. Although we cannot see this bond, the chemistry is sufficiently real that the bond can be represented as a thread (or a rung on the ladder). The pearls come in four different varieties — cytosine, thymine, guanine and adenine — each known by their first letter. C and T are slightly smaller than G and A.

Along the strands the pearls are held together by the chain, and the pearls can occur in any order at all. This is called the ‘sequence’ and it may read like this: CG GATTGAGCCTAGCTTTACCTAGAAC. However, the invisible bonds *across* the two strands are much more specialised. Not only does a large pearl link only with a small one, but C will only link with G, and A with T.

Despite the apparent randomness of the long sequences, knowing the pearl sequence of either strand makes the sequence of the other strand entirely predictable. This is because every pearl must link with the one opposite it, and will only link with its special partner.

In chemical terms, the smaller pearls are *pyrimidines* and the larger ones *purines*. T and C are pyrimidines; A and G are purines. The pyrimidines automatically seek out the purines, and vice versa.

The necklace is very, very long. In fact, in each chromosome there are between 100 million and 300 million pearl-pairs. Each of the two strands is known as a *nucleotide polymer* or a *nucleic acid*. Linked together, the two strands have the name *deoxyribose nucleic acid* or DNA. End to end, the double strand can be regarded as one molecule of DNA. And this one single molecule, with a few extra decorations, represents one *chromosome*. In some primitive life forms the ends would join because their chromosomes are circular.

In the genetic make-up of humans, there are 23 types of these double-stranded necklaces. In a fortunate vote for simplicity, they have been numbered from 1 to 23. Every person has two versions of each strand — one from mum and one from dad — giving a total of 46.

The necklaces have a natural tendency to twist, like streamers. More than that, these extremely long necklaces also have a tendency to ‘pack’, as if the streamers

were trying to condense themselves into shorter, thicker ropes. Theoretically, there are different ways in which the necklaces could pack, but one way seems to be the preferred option. It might be imagined thus.

In the nucleus, along with the 46 double-stranded necklaces, are some large 'beads'. They are all much the same, and they are complex. Each of them is actually made up of eight smaller protein pieces, wedged tightly together. This is called the histone core. Each of the eight pieces has a little 'tail' protruding. This makes the 'bead' look hairy, as eight little fronds protrude from every bead. These hairy beads, with their tails, are called histones; they are not part of the necklace as such, but the necklace seems to wrap naturally around them.

## DNA makes proteins

The whole purpose of DNA is to make proteins.

On the chromosome, bases or 'pearls' are strung along the necklace in a sequence that constitutes a highly specific code. Every set of three pairs codes for one *amino acid*. The next set of three codes for another, and so on. By reading the code, one triplet at a time, the mechanism exists for assembling amino acids, one by one, in the order dictated by the code. Amino acids, put together one by one in this manner, build up a *peptide*. Peptides put together build up a *polypeptide*, which is the basic unit of a *protein*. So if we 'read' along the necklace, we have the recipe for the different proteins, one by one.

A *gene* then, is a strip of the DNA with the code for a particular protein written on it. A slightly more formal definition is 'a nucleic acid sequence which codes for a functional polypeptide'. How many base pairs there are in any one gene depends on the complexity of the protein in question.

DNA is often referred to as a library, because of the information it contains. Under normal circumstances, the cell makes good use of this library, and is in no doubt about how, when and why its genetic material is read. It has good mechanisms for recognising where one gene ends and another starts, when one gene needs to be turned on, another off.

DNA is a library with a lot of activity. Books are being pulled off the shelf, consulted, photocopied and replaced all the time. However, there is one special feature about this library. Almost every cell in the organism contains the *whole* library, but depending on which cell it is, it only ever uses a small section of it.

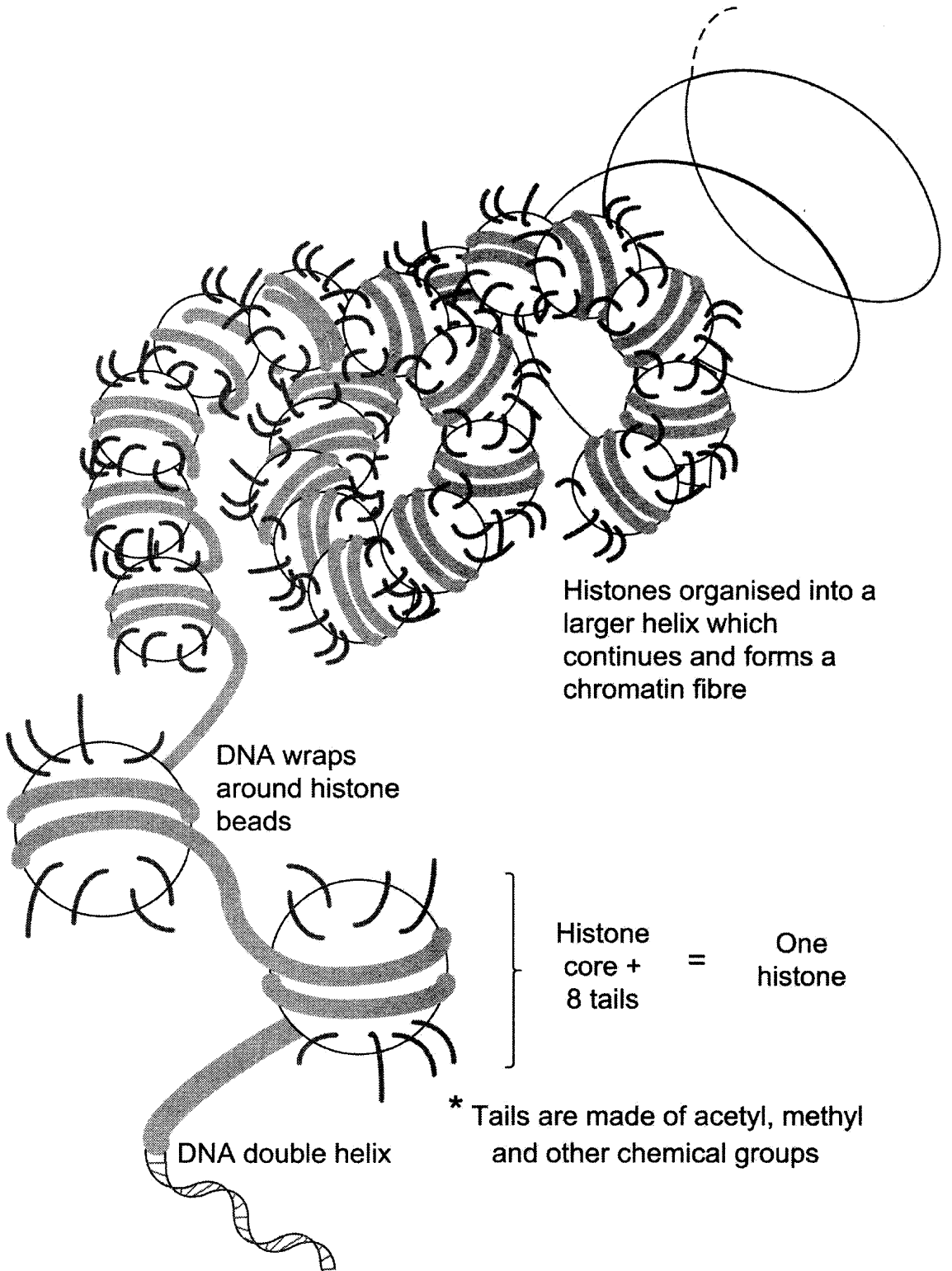


Chart 3.7: Histone beads

Thus a liver cell has not only all the instruction books it needs to operate as a liver cell, it also has the books on how to be a nerve cell, a heart cell, a kidney cell, and so on. It just never consults them. At least, that is what we think at this stage of understanding of genetics. (A stem cell, incidentally, is one which still is able to consult the whole library. It retains the ability to become any kind of cell. It has not yet become typecast.)

Notwithstanding this apparent wastage, the books that any one cell *does* need are in constant usage. When the cell needs to make a protein, either for its own use or to put into general circulation, it sends a messenger molecule into the nucleus. This molecule finds the right gene, and that bit of DNA unwinds. An enzyme in the nucleus (one of the librarians, let us say), unzips the gene for that protein, right down the middle, breaking the bond between the bases.

Floating around in the nucleus are other bases, sugars and phosphates, 'on call' as it were. These molecules attach themselves to the exposed edges of the zip, following the rules about base-to-base. The rules have altered just a bit. G still bonds to C, but A bonds not to T, but to another base called Uracil (or U), which is chemically very similar. Another difference is that the sugar that will be used this time is a *ribose*, not a *deoxyribose*. The 'photocopy' therefore is a slightly edited version of the original, and is called RNA rather than DNA.

At this stage the photocopy is just that: a blueprint of the instructions for making the protein. The enzyme, or librarian, then clips the photocopy off the original, and the newly made RNA (known as mRNA, 'm' for 'messenger'), travels back out of the nucleus and into the cytoplasm of the cell, where it 'docks' at a structure called a *ribosome*. The ribosome is a small and efficient factory, well equipped with the raw materials (amino acids) and personnel (transfer RNA, or tRNA) for reading the code and manufacturing the desired protein. Simple!

The cell is a constant production line, making the proteins that it needs to. Because the data are constantly accessed, the chromosomes are often in a state of undress in those places where they are unzipped.

## Cell division

Two types of cell division are possible: mitosis and meiosis.

- *Mitosis*: When cells in an organ have undergone some kind of damage,

or have simply worn out, a younger cell divides to replace the loss. The aim here is to ensure that new cells are as near as possible the same as the cells being replaced. All the chromosomes are unzipped from end to end, and attach to the available sugars, phosphates and bases in the nucleus. This is like the photocopy described above, but instead of just a part of the necklace (a gene), becoming unzipped, it is the whole necklace. Inevitably some mistakes are made during duplication. There is also a change in the length of the *telomeres*, the point at which the chromosomes pair up in cell division. These changes and mistakes are the basis of the ageing process.

- *Meiosis*: The other kind of cell division is by the formation of *gametes*, the reproductive cells (sperm and eggs). The genetic material is divided into halves during this process so the sperm and egg each carry only 23 chromosomes instead of the full set of 46. The chromosomes seek out their 'twin' (*non-identical*), known as the 'homologous chromosome'. The result of this process (and of some gene swapping as well), is that the maximum genetic mix is achieved, and the gamete gets a good mix of the characteristics of two grandparents. As the same thing happens in the formation of both sperm and egg, the chances are that the genes of all four grandparents are well represented in the resultant offspring.

## Genetic variation

If human chromosomes are so predictable, how come we all look different? The fact is that variations in the code, or gene, for a particular characteristic are not only possible, but essential. Otherwise, we would all be clones.

The differing versions of a particular gene result in a protein that is not quite the same as someone else's protein. But it will still do the job. This is most evident when you compare not individuals but species. The insulin molecule, for example, starts out as a polypeptide (protein) chain of about 108 amino acids. From species to species there are some minor variations in these amino acids, and thus, we can assume, some slight variations in the base pairs which coded for them. But human diabetics have used insulin from cows and pigs, with only minor problems.

When there are minor functional variations of a particular gene *within* a species, we say that there are different *alleles* of that gene. Or we say that the gene exhibits *polymorphism*.

If the variations do not matter, the different alleles will persist in the species. Some variations may even confer benefit. Skin colour is a good example. Prior to the mass migrations of the past few centuries, people who lived around the equator all had dark skin. This protected them from the harshness of the sun's rays. As people moved north, those who had lighter-coloured skin tended to survive better. Fair skin admitted adequate light for the manufacture of Vitamin D, giving these migrants an advantage (Chapter 5).

Some variations matter enormously. The alteration of a single amino acid can so distort the structure of the resultant protein that it can no longer do the job. The shape of the protein may be so critical that no variations are allowable. Such mutations either result in disease, or are altogether incompatible with life.

Variations in genes give us the vigour that enables a species to thrive. When an organism makes a 'mistake' during the process of gamete formation as a result of background radiation, chemical input or some other factor, then a new gene is created. If the new gene is deleterious to health, it may kill the person who carries it, and it will not be passed on. If it has some advantage, it may survive and flourish. Eventually it may become the most common allele.

Sometimes, although the new gene causes ill-health, it also confers some benefit and thus survives. The best example of this is the genes for thalassaemia and sickle-cell anaemia. Both of these genes are most prevalent in the areas where malaria is endemic. Malaria has killed up to half of all the people who have ever lived, so any gene which gives a defence against malaria has something going for it. The mutations which cause thalassaemia and sickle-cell anaemia distort the red blood cells in such a way that they can cause health problems for the person carrying those genes. However, the malaria organism finds it harder to parasitise these oddly shaped cells because they are less suitable for incubating its larval phase, which therefore does not thrive. Because the people carrying the genes gain partial protection from the scourges of malaria, those genes survive. Unfortunately, the people who get a double dose of the gene (one from each parent) have such distorted red cells that they often have shortened lives and much ill-health.

Cystic fibrosis provides another example. With increased understanding of genetics we came to realise that this disease was in fact many diseases, only one or two of which caused serious illness. Others caused minor problems, and some were not much different to the healthy alleles. Here again there is the suggestion of

benefit for the 5 per cent of Caucasians who carry just one copy of a defective allele. The resulting alteration in sodium and chloride metabolism not only produces salty sweat (a marker for cystic fibrosis), but also offers protection against diarrhoea, and therefore cholera.

Then there is the case of lipoprotein (a), which is discussed later as a possible contributor to heart disease. However, it appears that it may have protected us from scurvy (see Chapter 8: Cardiovascular disease).

There are about 30,000 genes in the human genome, but only a relative few are known for the trouble they cause. This is because some structures are inherently stable no matter what you do to them, and some are not. If you remove a brick from the top of a pile of bricks, the change will go unnoticed. Remove the keystone from an arch, and the whole arch will fall in. Depending on both the location of the amino acid and the *design of the original molecule*, a change in one or more of the amino acids will cause anything from 'no effect' through to catastrophe. Some proteins coded for by our genes are structurally robust, so slight alterations are of no consequence. Others are fragile; they can tolerate little alteration, or no alteration at all.

### The effects of 'bad' genes

A person who has only one copy of a particular allele (good or bad) is said to be *heterozygous* for that allele. A person who has two copies is *homozygous*. Often differing alleles have a blending effect; for instance, black skin and white usually results in an in-between skin colour. Genes that consistently over-ride the effects of other alleles are called dominant genes.

There is no doubt that we all have 'bad' genes. If the bad gene is dominant, it may be enough to make us sick. Mostly, we will have a 'good' gene from our other parent which will compensate for it. This is why many people with 'bad' recessive genes never know they carry such genes unless their spouse has the same recessive gene. Even then, the couple will need to have four children (statistically speaking) in order to give birth to an afflicted child who is unlucky enough to have a double dose of the bad gene.

But the terminology of 'good' and 'bad' reduces a complex situation to the level of a B-grade movie. It is true that some genes, both recessive and dominant, are inherently lethal, but in most cases the story is not black and white.

Enzymes are proteins, and the genes that code for enzymes often exhibit polymorphism. In fact, multiple variants, or alleles, exist for up to 30 per cent of all of the thousands of enzyme systems in the human body. Health problems can arise if any one of these enzymes has a malfunctioning variant. Problems can also arise when the enzyme exists in a slightly less efficient variant, or when production of the normal variant is slow. Slow production can be due to environmental factors, but it can also be built into the genetic code.

For instance, asthma may be due to a sluggish *delta-6 desaturation enzyme*. Delta-6 helps convert dietary precursors into anti-inflammatory prostaglandins (Chart 5.3).

But reduced efficiency only matters when demand outstrips supply. In a past era, most such challenges could be met. It took industrialisation to expose the enzyme systems with reduced robustness. Without the load of pollution, mineral deficiencies and other stress factors, many of these genetic variants might have caused no problems at all.

Rather than dismissing ailments as genetic, we need to understand how to get the best out of the genetic hand we have been dealt. Whether it is a question of our response to alcohol, or the chance of our 'cancer genes' giving us cancer, the fundamental issue is *what else* is going on in the cell to activate or suppress these genes.

To modify or limit the effects of genes there are three main areas of interest:

- Can the body adjust to a genetic weakness by some compensatory mechanism?
- What affects the expression of our genes?
- How do we avoid mutations (as these are more likely than not to be deleterious)?

### Compensatory mechanisms

Toxins include synthetic and natural poisons. Some, such as alcohol, aldehydes and alkaloids, occur naturally. Some are in our food. Some are produced by normal metabolism. Mother Nature has equipped us to deal with toxins.

One example is alcohol, which occurs widely in nature. There are wood alcohols, yeast alcohols, alcohols in ripe fruit, and so on. Retinol, also known as

Vitamin A, is an alcohol.

After consumption, ethanol is metabolised to acetaldehyde by enzymes known as *alcohol dehydrogenases*. (There are at least four of these alcohol dehydrogenases, but most detoxification is performed by Class I dehydrogenase, which occurs in the liver.) Then the mitochondria of the liver and muscle cells convert this acetaldehyde to acetic acid, with energy as a by-product. However, about 40 per cent of Japanese, Chinese and indigenous Americans, and a further 10 per cent of other people, lack the necessary enzyme, *aldehyde dehydrogenase*. This results in an accumulation of aldehyde (causing among other things, a red face and reduced alcohol tolerance). Such people are dependent on a much less efficient second pathway to deal with alcohol. This is known as *the oxidising pathway of the endoplasmic reticulum*. We all use this pathway when the first one is overloaded by excess consumption.

The issues here are several. All the dehydrogenases are metallo-enzymes, requiring two zinc atoms (Chapter 3). One zinc is structural and the other is required by the catalytic process. The conversion of acetaldehyde to acetic acid by aldehyde dehydrogenase requires the presence of molybdenum.<sup>12</sup> Both pathways require zinc, one requires molybdenum. They also consume Vitamin C and the B-group vitamins. The degree to which we react to alcohol is determined not only by the kind of enzymes we drew from the genetic lottery, but also by the vitamins and minerals in our diet.

Another example is high levels of homocysteine, recognised as a risk factor for cardiovascular disease. This problem seems to have a genetic base, and to run in families. A similar observation can be made about people with high cholesterol levels, despite low fat consumption. The genetic basis of these problems is well understood.

Raised plasma homocysteine is treatable with folic acid, Vitamin B6 and Vitamin B12. Raised cholesterol can be treated with Vitamin C, Vitamin B3, B5, and a whole range of dietary interventions.

Consumption of alcohol is a challenge to our metabolism, which has to work hard to detoxify it. The refined modern diet also acts as a challenge for those with any vulnerability in one or more of their enzyme systems. The natural consequence of this diet is to effectively ferret out our genetic weaknesses.

Another example is a condition known as spinal muscular atrophy (SMA),

which occurs in about one baby in 6000. It is the most common genetic cause of infant mortality worldwide. It causes muscle weakness and wasting, and most victims die before they reach the age of two. Yet some people with SMA live reasonably long lives. The illness is caused by a single mutation in a gene which is responsible for making a protein vital to the function of motor neurones. For this protein to be effective it needs to undergo methylation (Chapter 3). Once again, folic acid and Vitamin B12 are included in the list of important methylators. Current research suggests that the reason that some people live long lives with SMA is that they have diets naturally high in these nutrients.<sup>13</sup>

Even genetic disorders as ghastly as Huntington's chorea appear to affect different sufferers with differing degrees of severity. It has been suggested that both dietary and toxic influences can impact on the expression of such genes.

This is not to imply that the effects of mutations are to be seen only as the result of bad diets. But it is interesting to consider that, if even lethal genes may be affected by good diets, how many defects are defects only in the presence of a poor diet.

### Controlling the expression of genes

Do we have control over whether 'bad' genes are turned on in the first place? Are they indeed only bad when turned on inappropriately? Can they be switched off when their activity is damaging the cells or the organism? Can other genes be switched on to improve the situation? These questions form the focus of much exciting research.

Geneticists distinguish our *encoded* genetic sequences from our *expressed* genetic sequences. Many of our genes require constant expression — such as the genes for energy metabolism — or the function of the cell will simply cease. Sometimes, however, illness is the result of inappropriate expression of genes. This can happen in either direction. Genes may be on when they should be off, or off when they should be on.

The prevailing demands on the cell determine what genes will be 'read' or expressed at any one moment. They may be turned on or off to deal with toxins such as alcohol, prescription drugs or pollution, or to govern minute-by-minute running of the cell.

Genes are switched on and off by elements known as *transcription factors*, which

include steroids. Or the triggers can be proteins which bind directly to enzymes (polymerases), and thus increase the rate of transcription. Inappropriate transcription (Chapter 1) can result from a range of influences, including ionising radiation, noxious chemicals, circulating stress hormones or co-enzyme deficiencies.

It is possible that structural stress on the DNA causes it to unfold, resulting in automatic transcription. Even the electrical impact of free radicals may provoke inappropriate transcription.

If we return to our image of DNA as a library, like all libraries, it is only as good as the interpretation of the data by whoever does the reading. Books in any library can get damaged. They can be misinterpreted, over-interpreted, disregarded or lost, or simply irrelevant.

As a doctor I have a sinking feeling when a patient says that they expect to get arthritis, cancer or whatever, because it 'runs in the family'. There is no doubt that genetic weaknesses run in families. But except for particular genes, such as those which cause haemophilia, not all will lead to disease.

Suppose I have been told that I have a cancer gene (an *oncogene*), BRAC1 or BRAC2. What should I make of this? Do I join those women who have prophylactic mastectomies or oophorectomies (removal of both ovaries)? I might ask what evolutionary sense there is to have a gene like this in the population. I might wonder if I had been born at another time, another place, how serious a threat these genes might have posed me. I would certainly want to know what turns these genes on, and off, what influences the transcription factors.

## Junk genes

From the description of the genetic code earlier in this chapter, it would be easy to get a picture of base pairs all neatly aligned in a linear sequence. However, while many sequences code for genes, the total amount of DNA coding in this manner constitutes only about 5 per cent of all our genetic material. Traditionally, the remainder has been referred to as 'junk DNA'.

We believed that junk DNA consists of leftovers from our evolutionary past. Now we suspect that this genetic 'debris' is carrying out vital functions, which may include turning the coding genes on and off.

About one-third of our junk genes are known as 'jumping genes' or *transposons*.

They are thought to be ancient viral remnants of retroviruses which infected our ancestors. Their DNA can persist as part of the genome. Some of these viral remnants can reproduce themselves and insert themselves into other chromosomes, causing illnesses such as the 'hereditary' leukaemias. Other retroviruses may bring adaptive benefits with them.

### **Histone codes**

The 'beads' in the DNA necklace have turned out to be more complex than we thought. It appears that the proteins in the histone core have a code of their own. This new code may well explain a type of inheritance known as 'epigenetic'.

### **Epigenetic inheritance**

Epigenetic refers to characteristics that appear to be inherited, but do not appear to be encoded in the genome. 'Some researchers even suspect that this might be where nature and nurture converge, the route by which our environment, stress, toxic chemicals and the food we eat can modify and manipulate the message written in our genes.'<sup>14</sup>

There is a disturbing aspect of epigenetic inheritance: genes may be permanently switched on or off by environmental presences or absences, such that future generations will show the effects.

For example, vitamin and mineral deficiency during pregnancy gives rise to a greater risk of diabetes, obesity and cardiovascular disease in the offspring, independent of subsequent nutrition. Has the histone code of the infant been damaged by nutritional depletion? Will this be passed on to his or her children?

We know that there is a greater incidence of congenital abnormalities in babies conceived in test tubes. Cloned animals which appear normal at birth have greater vulnerability to infection; they are prone to dying suddenly from conditions such as organ failure or cardiovascular disease. It is perhaps too early to speculate on the causes of such problems, but it is reasonable to observe that, during the critical phases of fertilisation and embryogenesis, the complex nutrients from a mixed healthy diet might be difficult to duplicate in the culture medium. The embryos have the same set of genes as the mother, but we have to wonder about what is happening to the on-off switches of those genes.

Other evidence has come from work at the University of Sydney, which shows

that epigenetic inheritance may be one of the factors involved in the rising incidence of asthma and autism.<sup>15</sup> Another example is a study that has shown that the diet of a mother animal can permanently influence fur colour in the offspring.<sup>16</sup>

The mechanism by which this may occur is through the acetyl and methyl groups attached to the histone core, which can affect whether the gene is expressed or not. Enzymes inside the nucleus (such as *methyl transferase* and *deacetylase*) add methyl groups, remove acetyl groups and so on. Researchers have identified at least 20 or 30 such enzymes with the potential to affect gene transcription in this way. The implication of this is that these dietary nutrients can determine the transcription of individual genes through gene silencing or activation.

Viral infection has been shown to trigger a series of acetylation reactions which turn on the gene responsible for the transcription of beta feron, a powerful anti-viral. Hormones have been shown to trigger gene expression via acetylation reactions. Many illnesses occur when cells act autonomously, or when genes ignore the modifying instructions of other genes. The histones provide a mechanism whereby 'bad' genes can be controlled. The methyl and other groups come from things as simple as the folic acid in leafy green vegetables.

It has been proposed that the gut bacteria may have a role in determining how genes are read. Here, too, the influence may come via modification of the histone code. Gut bacteria, as previously discussed, produce short-chain fatty acids, or SCFAs, including butyric acid. Butyric acid has been demonstrated to be a potent inhibitor of histone de-acetylases.<sup>17</sup> A deficiency of butyrate as a result of a reduction in gut flora may, in theory at least, allow an enzyme to switch on an 'oncogene', which is then inappropriately expressed.

Plant and animal cells seem to be able to block a specific gene by destroying the RNA copies made by that gene. This is called RNA interference, or RNAi. There has been some excitement in the research world about the potential for many illnesses to be treated by blocking the relevant genes in this way. The approach has been to add small matching pieces of interfering RNA (RNAi) to the cell. So far the results have indicated that there is collateral damage to adjacent genes, which is, of course, undesirable. Perhaps the most reliable technique for the moment is to ensure that the cell has all the nutrients it needs to support its own targeting process.

## Avoiding mutations

When a gene is damaged, several things can happen. The cell itself may recognise that the damage is beyond repair and commit suicide. Within the genetic material are genes which contribute to this process.

Often the damage is recognised by the very enzymes the DNA itself has produced, and these enzymes will set about repairing that damage. The enzymes, called *DNA repairase*, are in constant use. Mutations occur when these enzymes fail to do their job. As the enzymes are dependent on co-factors such as selenium and zinc, it is clear that a deficiency of such co-factors compromises the ability of DNA to repair itself.

If repair has not taken place, and the cell has not destroyed itself, then the result is that a permanent change has taken place in the code, and this is called a *mutation*.

A mutation that occurs in a germ cell (eggs or sperm) is passed on to the next generation. If it occurs in a somatic cell (that is, any cell in the body which is not a germ cell), the damage is confined to that cell and its daughter cells. It may be of no consequence at all. On the other hand, it may be the first step in the process of that cell turning into a cancer cell.

Although we have many defences against mutations, prevention is always better than cure. Avoiding cigarettes, radiation, pollution and chemicals is one half of this equation. But we should be quite clear about one thing: 'Most genetic mutations are caused by free radicals scavenging electrons from DNA.' This is nothing more nor less than oxidative damage.<sup>18</sup> Therefore, eating a good diet rich in anti-oxidants and leading a healthy lifestyle is the other half.

We cannot blame our genome, millions of years in the making, for the diseases of Western civilisation. We have significant control over most of our bad genes. Although at this stage much is still in the research phase, we may arrive at the conclusions our grandparents never questioned: that sunshine and exercise are good for us; that we need fresh air and adequate rest; that we are what we eat.