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Genetic engineering for medicine

"Breakthrough" is a word studiously avoided by most doctors, researchers, and respectable journalists; but Professor John Paul from Glasgow used it recently to describe genetic engineering when addressing the Society for Drug Research. He is probably right. Genetic manipulation, which has mostly developed only in the past decade, is the process of changing the genetic material of a cell: genes (sequences of DNA) from one cell—for example, a human cell—can be inserted into another cell—often a bacterium—and made to function. Already with these techniques human insulin, growth hormone, and interferon have been produced by bacterial cells. Moreover, insights have been gained into fundamental cell functions, the behaviour of tumour viruses, and the mechanisms of some genetic diseases. These new ideas have influenced all kinds of biological work; and their impact will soon reach the general practitioner, who should be able to prescribe human insulin made by bacteria.

Although the techniques are dauntingly complex, the principles of genetic engineering are simple enough for any doctor to understand. There are three problems to be overcome: firstly, to prepare the appropriate genes; secondly, to insert them into the "effecting" organism, so that they replicate as the organism reproduces; and, thirdly, to ensure that the inserted genes are expressed—that is, that the effecting organism actually makes the coded protein. Segments of DNA for insertion can be prepared by breaking long chains into smaller pieces by the use of restriction enzymes, naturally occurring enzymes that cleave the DNA chains at defined points. Alternatively, small genes can be synthesised: nucleic acids can be combined in the laboratory, at the moment for a length of up to about 100 of the constituent bases, or small lengths of DNA can be synthesised from templates of messenger RNA with the help of reverse transcriptase (the enzyme produced by some tumour viruses that can make DNA from RNA instead of vice versa). The segments are inserted into the effecting organism—usually *Escherichia coli*—by using plasmids and bacteriophages.

Plasmids are small packets of DNA that are found within bacteria and can be passed from one bacterium to another; they are best known to doctors for their ability to transfer antibiotic resistance. By using appropriate enzymes, sections of foreign DNA can be inserted into the DNA contained in the plasmid. Plasmids pass into the bacterial cell and, while remaining separate from the gene complement, they then use the cellular machinery to manufacture proteins. Another way of inserting the foreign DNA into bacteria is by first inserting it into a virus. Various techniques are available to select out those bacteria carrying the required inserted genes (for instance, genes for human insulin), and to encourage the bacteria to replicate and make the required protein in high concentration.

The molecular biologists, who have devised all these techniques, have been the first to benefit from their own work. Genetic engineering is producing many new insights into the workings of the genes of both prokaryotic cells (cells such as bacteria with no true nucleus) and eukaryotic cells (those, such as animal and plant cells, with a true nucleus). Many of the genetic mechanisms of prokaryotic cells had been worked out before genetic engineering was developed, and molecular biologists had tended to assume that eukaryotic cells would be similar. Genetic engineering has helped to show that this assumption was false: as Dr R A Flavell from the National Institute for Medical Research said at the conference, the more molecular biologists look at the two types of cells the more differences they discover. The beauty of this fundamental research is that as genetic engineering teaches us more about the cell so the new understanding can advance the techniques of genetic engineering. One fundamental problem with clinical overtones now ripe for solution is that of tumour viruses: why do tumour viruses which can be quiescent in animal cells suddenly turn them malignant?

More pressing to doctors (and patients) are the problems of genetic diseases. Genetic engineering may have much to contribute to these problems in increasing understanding of the mechanisms, in improving methods of diagnosis (particularly antenatal diagnosis), and perhaps in actually treating some of these diseases. At the conference Professor David Weatherall kept his feet firmly on the ground and illustrated the possibilities using the thalassaemias as an example. At the moment β-thalassaemia can be diagnosed antenatally only by obtaining a sample of fetal blood at about 15 weeks' gestation. This demands fetoscopy and fetal blood sampling, which in the best hands carries a fetal mortality rate of about 1-2% and often in routine practice of about 5% or more. It had been hoped that gene maps of fetal DNA (which can be obtained by amniocentesis) would allow safer and easier diagnosis. Sadly, the gene maps look the same in most patients

with β-thalassaemia as in normal individuals. Encouragingly, however, new techniques for breaking up the genes by the use of enzymes may allow the genes of patients with β-thalassaemia to be distinguished from normal genes. But this work is at an early stage and is applicable for the diagnosis of β-thalassaemia only in certain populations. More encouraging is the recent observation that this approach can accurately identify fetuses homozygous for sickle-cell anaemia.

The production of human proteins is the first tangible benefit from genetic engineering. One drug company is already testing in patients human insulin produced from bacteria, and other products are in the pipeline. Dr D Denner, of Eli Lilly Company, who has experience of trying to produce insulin on a commercial scale, thinks that the prospects for producing drugs by genetic engineering methods lie in making larger quantities of familiar hormones and enzymes rather than new drugs. Nevertheless, Professor D Hopwood from Norwich thought that genetic engineering could help eventually in developing new antibiotics. He pointed out that, unlike hormones and enzymes, antibiotics were not pure gene products but metabolites resulting from reactions with many different steps. But many of the techniques applied to *E coli*—the usual model of molecular biologists—could be applied to streptomycetes, the micro-organisms that produce most of our present antibiotics; and new antibiotics might eventually result.

One problem that overhangs this exciting work is that of regulation. In the early days of genetic engineering governments and scientists were worried that something dreadful—such as bacteria resistant to all antibiotics, or cancer-causing organisms—might result from this tinkering with the very stuff of life. Consequently, strict regulations were evolved: there were elaborate rules on physical and biological containment (biological containment consists in manipulating bacteria in such a way that they cannot survive outside controlled conditions in the laboratory). But, as Professor John Paul observed, the dangers are now seen to be those of the organism that is being used—thus elaborate precautions are essential for containing the smallpox virus but not *E coli*. Controls are now being relaxed; but he did point out that agreements are needed to limit the use of genetic engineering in making biological weapons. Let us hope that this "biological breakthrough" is not used for harm as the "atomic breakthrough" was. Even for the medical uses of genetic engineering, the complex ethical questions should be answered before and not after the techniques have been developed.

Primary pulmonary hypertension

Primary pulmonary hypertension, first described clinically by Paul Wood,[1] is a rare, progressive, and usually fatal disease which is more common in women.[2–4] The diagnosis can be made only by excluding the many causes of secondary pulmonary hypertension, starting with left ventricular failure, remembering aortic stenosis, and working back through mitral valve disease and left atrial tumour to congenital anomalies of the pulmonary veins, including cor triatriatum and supravalvar stenosing ring. Precapillary pulmonary hypertension may conceal congenital septal defects. Rarely fibrosing aveolitis or advanced sarcoidosis may present with severe pulmonary hypertension. In many tropical countries pulmonary bilharziasis closely simulates primary pulmonary hypertension, but in Britain pulmonary thromboembolism and veno-occlusive disease are the main differential diagnoses.

At the extremes of age the sex incidence is roughly equal, but most patients with primary pulmonary hypertension are women in the childbearing years. Pulmonary hypertension is usually severe by the time symptoms develop. Patients frequently present with syncope on effort. All of them are breathless, and they tend to show slight cyanosis. Considerable central cyanosis may be seen in a few patients with advanced disease who may shunt blood from the right to the left side of the heart if the foramen ovale is patent; peripheral cyanosis may be severe in patients with a very low cardiac output.

The disorder may be missed until far advanced because the physical signs are sometimes subtle and hard to elicit—even by the expert unless aided by the electrocardiogram and chest radiograph. Regular rhythm is usual; a giant venous *a* wave in the neck may be provoked but is often absent at rest; and there may be a parasternal heave, a right atrial beat, and a loud, even palpable, pulmonary closure sound. An ejection click can usually be heard at the left sternal edge if the patient holds his breath in expiration. Splitting of the second sound with respiration remains normal until the right ventricle starts to fail, when pulmonary closure becomes delayed in relation to a relatively early aortic valve closure. A diastolic murmur of pulmonary regurgitation and a murmur of tricuspid regurgitation, often misleadingly loud, may appear. The electrocardiogram usually shows sinus rhythm with right axis deviation and almost invariable T wave inversion in the right-sided chest leads. Voltage changes indicating right ventricular hypertrophy vary from slight to extreme, probably reflecting the duration of the disease. Chest radiographs often show a normal sized heart but with a dilated main pulmonary artery trunk and perhaps increased transradiancy of the lung fields caused by narrowing of the peripheral branches of the pulmonary artery. Later on in the illness the right heart chambers dilate, but sudden death may occur before heart failure develops.

Exercise tolerance is appreciably diminished with an increased pulse-to-work ratio, hyperventilation, and often a fall in blood pressure (the stroke volume does not rise to maintain blood pressure in the face of vasodilatation in exercising muscles)—the usual mechanism of syncope. Loss of consciousness may mistakenly be attributed to secondary cardiac arrhythmias; even epilepsy has been mimicked in some cases.

The echocardiogram is useful in excluding silent mitral stenosis as well as in showing a normally contracting left ventricle and dilatation of the right ventricular outflow tract. There may be increased thickness of the ventricular septum and of the anterior right ventricular wall as well as absence of the *a* wave in the pulmonary valve echo and an increased pulmonary ejection time shown by prolonged opening of the pulmonary valve. Prolapse of the mitral valve is occasionally seen; it may be attributed to the diminished size of the "starved" left ventricle. Cardiac catheterisation shows a high pulmonary artery pressure, frequently at or about systemic level when the disease is first recognised. A pulmonary wedge pressure reading may be difficult to obtain but this or the directly measured left atrial pressure is normal. The cardiac output is usually low. Pulmonary angiography shows normal anatomy but with dilated proximal branches, and macroangiograms may show the peripheral attenuation of small vessels—which accounts for the blackness of the lung fields in *x*-ray films.