than those in consultant units.²² The policy of the Department of Health, however, remains that recommended by the Peel report,²³ and repeated by the Short report in 1980²⁴: facilities should be provided to permit hospital delivery in all cases.

This story of the happy pursuit of a policy unsupported by sound evidence is a sorry reflection on the political process. It has particular relevance now, when general practitioners have been offered a contract obliging them to do yearly check ups on all patients aged over 75.

The low water mark of general practice obstetrics may yet have been reached. At the recent meeting in Birmingham, which decided to set up an independent body to support general practice obstetrics (*BMJ* 11 March, p 626), doctors were told of some consultant obstetricians' problems in filling junior obstetric posts. Such gaps could be filled by general practitioners. Doctors at the meeting took as another encouraging sign the government's commitment to consumer choice—although obstetrics is not mentioned in *Working for Patients*,²⁵ the government should support a topic where real choices can be made.

Whether the meeting will be perceived to be the beginning of the rebirth of general practitioner obstetrics or the first preparation for the obsequies remains to be seen.

DAVID JEWELL

Consultant Senior Lecturer in General Practice,
Department of Epidemiology and
Community Medicine,
Bristol University,
Bristol BS8 2PR

- 1 Taylor GW, Edgar W, Taylor BA, Neal DG. How safe is general practitioner obstetrics? *Lancet* 1980;ii:1287-9.
- 2 Wood LAC. Obstetric retrospect. *J R Coll Gen Pract* 1981;31:80-90.
- 3 Klein M, Lloyd I, Redman C, Bull M, Turnbull AC. A comparison of low-risk pregnant women booked for delivery in two systems of care: shared-care (consultant) and integrated general practice unit. I. Obstetrical procedures and neonatal outcome. *Br J Obstet Gynaecol* 1983;90:118-22.
- 4 Klein M, Lloyd I, Redman C, Bull M, Turnbull AC. A comparison of low-risk pregnant women booked for delivery in two systems of care: shared-care (consultant) and integrated general practice unit. II. Labour and delivery management and neonatal outcome. *Br J Obstet Gynaecol* 1983;90:123-8.
- 5 Cavenagh AJM, Phillips KM, Sheridan B, Williams EMJ. Contribution of isolated general practitioner maternity units. *Br Med J* 1984;288:1438-40.
- 6 Shearer JML. Five year prospective survey of risk of booking for a home birth in Essex. *Br Med J* 1985;291:1478-80.
- 7 Young G. Are isolated maternity units run by general practitioners dangerous? *Br Med J* 1987;294:744-6.
- 8 Lowe SW, House W, Garrett T. Comparison of outcome of low-risk labour in an isolated general practice maternity unit and a specialist maternity hospital. *J R Coll Gen Pract* 1987;37:484-7.
- 9 Garrett T, House W, Lowe SW. Outcome of women booked into an isolated general practitioner maternity unit over eight years. *J R Coll Gen Pract* 1987;37:488-90.
- 10 Black N. Do general practitioner deliveries constitute a perinatal mortality risk? *Br Med J* 1982;284:488-90.
- 11 Barron SL, Thomson AM, Philips PR. Home and hospital confinement in Newcastle upon Tyne, 1960-1969. *Br J Obstet Gynaecol* 1984;84:401-11.
- 12 Campbell R, MacDonald Davies I, MacFarlane A, Beral V. Home births in England and Wales, 1979: perinatal mortality according to intended place of delivery. *Br Med J* 1984;289:721-4.
- 13 Murphy JF, Dauncey M, Gray OP, Chalmers I. Planned and unplanned deliveries at home: implications of a changing ratio. *Br Med J* 1984;288:1429-32.
- 14 Tew M. Place of birth and perinatal mortality. *J R Coll Gen Pract* 1985;35:390-4.
- 15 Tew M. Do obstetric intranatal interventions make birth safer? *Br J Obstet Gynaecol* 1986;93:659-74.
- 16 Rosenblatt RA, Reinken J, Shoemack P. Is obstetrics safe in small hospitals? Evidence from New Zealand's Regionalised Perinatal System. *Lancet* 1985;ii:429-32.
- 17 Drife JO. My grandchild's birth. *Br Med J* 1988;297:1208.
- 18 Campbell R, MacFarlane A. *Where to be born? The debate and the evidence*. Oxford: National Perinatal Epidemiology Unit, 1987.
- 19 Lilford RJ. Clinical experimentation in obstetrics. *Br Med J* 1987;295:1298-300.
- 20 Taylor A. Maternity services: the consumer's view. *J R Coll Gen Pract* 1986;36:157-60.
- 21 Damstra-Wijmenga SMI. Home confinement: the positive results in Holland. *J R Coll Gen Pract* 1984;34:425-30.
- 22 Stilwell JA. Relative costs of home and hospital confinement. *Br Med J* 1979;ii:257-9.
- 23 Standing Maternity and Midwifery Advisory Committee. *Domiciliary midwifery and maternity bed needs*. London: HMSO, 1970.
- 24 Social Services Committee. *Perinatal and neonatal mortality*. London: HMSO, 1980. (Second report from the Social Services Committee, session 1979-80, vol 1.) (Cmd 663-1.)
- 25 Secretaries of State for Health, Wales, Northern Ireland, and Scotland. *Working for patients*. London: HMSO, 1989. (Cmd 555.)

Gene therapy

Getting there slowly

In a subject that is moving so quickly that the transatlantic telephone and fax machine are essential for survival gene therapy seems to have been just round the corner for an embarrassingly long time. Current research is directed towards somatic gene therapy—that is, the transfer of genes into cells other than those of the germ line. The alternative approach, germ line therapy, in which genes are inserted into fertilised eggs and so passed on to future generations, though already well advanced in animals, is not being contemplated in humans.

Before genes can be inserted into patients several pre-requisites must be fulfilled. Firstly, to counsel prospective recipients and their families we must be able to give an accurate account of the clinical consequences of the illness—genetic diseases vary widely in their severity, and even when their molecular basis is known it is not yet always possible to give an accurate prognosis. Secondly, we have to isolate the particular gene and define the regulatory regions that are responsible for its expression in appropriate amounts in the right tissues. Thirdly, we must identify and isolate the correct target cells and develop efficient and safe methods with which to introduce the gene. Finally, unequivocal evidence must be available from animal experiments that the inserted gene functions adequately and produces no deleterious effects in its new environment and that the recipient cell population has an adequate life span.

Bone marrow is accessible, and sickle cell anaemia and thalassaemia were the first monogenic disorders to be

characterised at the molecular level, so it is not surprising that they became the first candidates for gene therapy. But there were formidable difficulties to overcome.^{1,2} Suppose, for example, that we wished to replace a defective β globin gene in a child with β thalassaemia. Firstly, we would have to find the target cell. As most red cell precursors in the bone marrow are already terminally differentiated (and so have a life span of only a few months) the gene would have to be inserted (transfected) into self renewing haemopoietic stem cells, a population that we cannot identify, which probably makes up only 0.1-0.5% of bone marrow cells. Secondly, even if we were fortunate enough to transfect some of them there is no reason why these stem cells should proliferate at the expense of their fellows; it is only their erythroid offspring that would benefit from the new gene. Thirdly, we would have to ensure that the inserted gene directs the synthesis of sufficient β chains to match the number of α globin chains in the same cell; if it produced too many it would convert the patient's β thalassaemia into α thalassaemia, not the therapeutic tour de force we had hoped for. And how could we be sure that the inserted genes would be expressed only in red cells; what would happen if they did so in white cells or platelets?

It was thoughts such as these and lack of progress in understanding how genes are regulated that changed the direction of research in gene therapy to disorders due to defective "housekeeping" genes. These are genes expressed at a low level in most cells, and they may (we hope) not be subject to such tight regulation as the more specialised genes

that control globin synthesis.² Recently, much effort has been directed at transferring genes such as those coding for adenine deaminase or hypoxanthine guanine phosphoribosyltransferase, defects of which are responsible for severe combined immunodeficiency and the Lesch-Nyhan syndrome, respectively.

Genes may be inserted into cells by injection into the nucleus, by encapsulation within lipid vesicles,³ by calcium phosphate mediated transfection,⁴ or by exposing the cells to a pulsed electrical field.⁵ As none of these methods has yet proved to be particularly efficient delivery systems are being developed, the most promising of which make use of retroviruses.⁶ These agents, adapted by evolution to deliver their genome into cells, have a complicated structure and lifestyle. The virion consists of a dimer of RNA with a protein coat surrounded by a lipid bilayer that contains specific glycoproteins that attach themselves to cells during infection. Once inside the cell the coat is shed and the RNA genome is copied into DNA by the viral enzyme reverse transcriptase. Specific retroviral sequences then direct the integration of the viral DNA into the host genome.

Various ingenious recombinant retrovirus vectors for gene transfer have been constructed.⁶ The proviral DNA needed for infection, integration, and transcriptional control of its genome, which are all contained in long terminal repeat sequences, are preserved together with the packaging sequence. The viral sequences for reverse transcriptase, internal structural proteins of the virion core, and the envelope glycoproteins are deleted and replaced by a dominant selectable marker together with the particular gene that is to be transferred. The recombinant and now defective retroviral genome, in the form of plasmid DNA, is then introduced into murine fibroblasts to generate cell lines that produce the recombinant retrovirus. Simultaneous infection with a wild type helper virus that has had its packaging sequence removed provides the required packaging proteins. Alternatively, a specialised packaging cell line that contains helper virus sequences within its genome can be used. In essence, engineered retrovirus vectors use their packaging sequences to package proteins produced by the helper virus, which, because it lacks these sequences, remains trapped in the cells. Recombinant vector virus particles are shed into the surrounding media, which is then harvested and used to infect recipient bone marrow cells—or the latter are incubated directly with cells that are budding off viral particles.

Clearly, gene transfer with recombinant retroviruses is feasible. Genes inserted into cultured haemopoietic cells or other cells may be expressed at levels similar to or greater than those of the endogenous genes.⁷ For example, the activity of hypoxanthine guanine phosphoribosyltransferase has been restored to a lymphoblast line derived from a patient with the Lesch-Nyhan syndrome and the metabolic defect almost completely corrected.⁸ In vivo studies have, however, been less encouraging. Recombinant retroviruses containing selectable markers have been introduced into murine bone marrow, which was then injected into lethally irradiated mice. By following the fate of the marked genes in colonies in the spleen they were shown to have been transferred into pluripotential haemopoietic stem cells.⁹ On the other hand, transplantation of transfected haemopoietic cells into mice or monkeys has usually been followed by a disappointingly low expression of the inserted genes and the population of blood cells containing the new genes has not survived long.^{10 11} Furthermore, there are still concerns about the safety of retrovirus transfection vectors. Integration into the genome is random and it is possible, though unlikely, that if the new gene becomes integrated in the wrong place it could cause serious side effects—activating an adjacent oncogene, for

example. And there is always the fear that recombination might lead to the generation of an infective retrovirus in the host.

But there are also reasons for optimism. Recently sequences have been identified in the human β globin gene cluster that when put in apposition to β globin genes and injected into fertilised mouse eggs direct the synthesis of large amounts of human β globin chains in the red cells of the transgenic progeny.¹² Furthermore, major technical improvements have been made in engineering retroviral vectors, leading to the development of built in safety features that should make unwanted recombination much less likely.^{6 13} The main problem that remains is how to transfer genes into the long lived haemopoietic stem cell population, which seems to be resistant to transfection, probably because much of it is out of cycle at any one time. Encouraging results have been obtained by transfecting other cell types—for example, fibroblasts.¹⁴ These cells might maintain more persistent expression of transferred genes, particularly as they undergo little differentiation after engraftment. This approach may make it feasible to transfer genes for blood clotting factors—for example, factors VIII and IX. Gene transfer predicted by a retrovirus has also been achieved in primary cultures of mouse and rat liver cells.¹⁵⁻¹⁷

Some recent successes have also been recorded in the targeted modification of genes by site directed recombination, a technique that has been used in yeast for many years but has only recently been applied to murine and human genomes.¹⁸⁻²⁰ Here the idea is that the exogenous DNA should contain a region with the same nucleotide sequence as the target gene so that homologous recombination can occur between regions of identical sequence. In other words, it simulates nature's way of gene mixing. Currently this approach is still inefficient and tends to generate new mutations, but in the long term it could be the most direct way of replacing defective genes. It has the particular appeal of replacing directly the defective sequence rather than inserting a new gene at random.

Molecular biology seems, therefore, to be getting there, but there are still formidable difficulties in cell biology to be overcome. The first priority is to correct a relatively straightforward disorder such as a defect in a housekeeping gene or a haemoglobinopathy. But because of the serious developmental defects associated with many genetic diseases—for example, the Lesch-Nyhan syndrome—in the long term it will be necessary to learn how to correct genetic defects early during human development. Doctors who are concerned about the risks and ethics of gene therapy can be reassured that it will only be applied in clinical practice when the safety criteria set out by the European Research Councils and National Institutes of Health have been met. In fact, somatic cell gene therapy raises no new ethical problems; it is no different in principle to organ transplantation. But we have to be sure that it is safe and effective before it is applied in clinical practice.

D J WEATHERALL

Nuffield Professor of Clinical Medicine,
MRC Molecular Haematology Unit,
Nuffield Department of Clinical Medicine,
John Radcliffe Hospital,
Oxford OX3 9DU

1 Weatherall DJ. *The new genetics and clinical practice*. 2nd ed. Oxford: Oxford University Press, 1987.

2 Anderson WF. Prospects for human gene therapy. *Science* 1984;226:401-9.

3 Mannino RJ, Gould-Fogerite S. Liposome mediated gene transfer. *BioTechniques* 1988;6:682-90.

4 Chen CA, Okayama H. Calcium phosphate-mediated gene transfer: a highly efficient transfection system for stably transforming cells with plasmid DNA. *BioTechniques* 1988;6:632-8.

5 Andreason GL, Evans GA. Introduction and expression of DNA molecules in eukaryotic cells by electroporation. *BioTechniques* 1988;6:650-60.

6 Eglitis MA, Anderson WF. Retroviral vectors for introduction of genes into mammalian cells. *BioTechniques* 1988;6:608-14.

- 7 Miller AD, Palmer TD, Hock RA. Transfer of genes into human somatic cells using retrovirus vectors. *Cold Spring Harbor Symp Quant Biol* 1986;51:1013-20.
- 8 Willis RC, Jolly DJ, Miller AD, *et al*. Partial phenotypic correction of human Lesch-Nyhan (hypoxanthine-guanine phosphoribosyltransferase-deficient) lymphoblasts with a transmissible retroviral vector. *J Biol Chem* 1984;259:7842-9.
- 9 Williams DA, Orkin SH, Mulligan RC. Retrovirus-mediated transfer of human adenosine deaminase gene sequences into cells in culture and into murine hematopoietic cells in vivo. *Proc Natl Acad Sci USA* 1986;83:2566-70.
- 10 Dzierzak EA, Papayannopoulou T, Mulligan RC. Lineage-specific expression of a human β -globin gene in murine bone marrow transplant recipients reconstituted with retrovirus-transduced stem cells. *Nature* 1988;331:35-41.
- 11 Anderson WF, Kantoff P, Eglitis M, *et al*. Gene transfer and expression in nonhuman primates using retroviral vectors. *Cold Spring Harbor Symp Quant Biol* 1986;51:1065-72.
- 12 Grosveld F, van Assendelft GB, Greaves DR, Kollias G. Position-independent high-level expression of the human β -globin gene in transgenic mice. *Cell* 1987;51:975-85.
- 13 Yee J-K, Jolly DJ, Moores JC, Respass JD, Friedman T. Gene expression from a transcriptionally disabled retroviral vector. *Cold Spring Harbor Symp Quant Biol* 1986;51:1021-6.
- 14 Anson DS, Hock RA, Austen D, *et al*. Towards gene therapy for haemophilia B. *Mol Biol Med* 1987;4:11-20.
- 15 Ledley FD, Darlington GJ, Hahn T, Woo SLC. Retroviral gene transfer into primary hepatocytes: implications for genetic therapy of liver-specific functions. *Proc Natl Acad Sci USA* 1987;84:5335-9.
- 16 St Louis A, Verma IM. An alternative approach to somatic cell gene therapy. *Proc Natl Acad Sci USA* 1988;85:3150-4.
- 17 Wilson JM, Jefferson DM, Chowdhury JR, Novikoff PM, Johnston DE, Mulligan RC. Retrovirus-mediated transduction of adult hepatocytes. *Proc Natl Acad Sci USA* 1988;85:3014-8.
- 18 Gregg RG, Smithies O. Targeted modification of human chromosomal genes. *Cold Spring Harbor Symp Quant Biol* 1986;51:1093-100.
- 19 Doetschman T, Maeda N, Smithies O. Targeted mutation of the Hprt gene in mouse embryonic stem cells. *Proc Natl Acad Sci USA* 1988;85:8583-7.
- 20 Mansour SL, Thomas KR, Capecchi MR. Disruption of the proto-oncogene int-2 in mouse embryo-derived stem cells: a general strategy for targeting mutations to non-selectable genes. *Nature* 1988;336:348-52.

PET scanning

Provides information on function

The acronym PET stands for positron emission tomography. This uses the tomographic principles of radiation detection and subsequent image reconstruction, but it is more than an imaging technique. Positron emission tomography measures local tissue concentrations of radioisotopes in the body and differs from tomographic imaging with techniques such as computed tomography or magnetic resonance imaging in that it provides functional information.

The versatility of this approach is shown by the wide range of variables that have already been measured and potentially can be measured in humans.^{1,2} Local tissue perfusion, blood volume, glucose and oxygen consumption, and fractional extractions of various metabolites and substances have all been quantitated and imaged with accuracy. In this, the use of a positron emitting isotope as a tracer is crucial. Radioactive forms of oxygen, nitrogen, and carbon can be made and substituted for the same stable elements in the molecules to be studied, thus avoiding alterations of the normal metabolic rate after introduction into the body that may result from the use of "foreign" isotopes such as iodine or technetium. The activity passing through a volume of scanned tissue can then be measured.

The variety of functions that can be assessed and imaged is limited by the availability of tracers that can be rapidly labelled chemically with the isotopes, which have extremely short half lives (for example, the half life of oxygen-15 is 2.1 minutes and that of carbon-11, 20 minutes). Measuring the amount of a labelled molecule in the tissues depends on several chemical, physical, and biological factors, any of which may be the focus of primary, clinical, or biological interest. The development of new PET tracer techniques depends on isolating this focus.

Measuring the oxygen and glucose metabolism has been widely used to investigate cerebral function and disease during the 10 years that PET has been used in humans. The brain uses these substrates as the sole providers of metabolic energy, and hence the consumption of glucose or oxygen, or both, reflects total neuronal function. Techniques have been used to highlight areas of the brain that participate specifically in performing motor, verbal, visual, and other tasks.³ As the methods have become more complex so rapid measurements, which can be repeated at the same session, are now possible, permitting complex analyses of neuropsychological functions and of responses to external stimuli.⁴ Metabolic measurements are also valuable in delineating areas of the brain with disturbed function—for example, the abnormalities of parietal and posterior temporal metabolism described in patients with early Alzheimer's disease.⁵ Another example is identifying

subjects at risk of Huntington's disease by finding low energy metabolism in the caudate nucleus. This pattern is universal in the established disease and its finding in apparently healthy people has raised the possibility of presymptomatic identification of gene carriers.⁶ Conversely, raised metabolism is found during pathological excitation, such as in epilepsy. PET has been found clinically useful in assessing patients with partial complex epilepsy for treatment with temporal lobectomy by detecting hypometabolic areas in a temporal lobe during the interictal period.⁷⁻⁹

Measuring haemodynamic variables such as tissue perfusion and blood volume together with oxygen metabolism has helped our understanding of ischaemic and pre-ischaemic states¹⁰⁻¹² and is being developed further for evaluating haemodynamic compromise in patients with stenotic and occlusive disease of arteries in the neck. This ability to measure and image hypoxia and ischaemia is also proving useful in studying myocardial ischaemia¹³ and the local physiology of tumours of the brain and other organs.^{14,15} More recently, methods have been introduced to measure the function of neurotransmitter systems in Parkinson's disease,¹⁶⁻¹⁸ schizophrenia,¹⁹⁻²¹ and partial complex epilepsy.^{22,23} Other tracers are being developed for the serotonergic system.²⁴⁻²⁸ In a few cases tracers have already been used for imaging and measuring the function of both presynaptic and postsynaptic components of a neurochemically distinct pathway.

The scope for labelling a wide range of chemicals and pharmaceuticals is immense. Recent advances suggest that further tracers will be identified and new methods developed in the coming years, while the performance of the PET scanners will probably also improve. The combination of metabolic and transmitter specific tracers is particularly attractive for investigating neurological disease, particularly the degenerative disorders of the deeper cerebral structures. The prospect of being able to classify in life some degenerative diseases presenting as dementias or akinetic-rigid syndromes will allow us to study their natural course and possible treatments.²⁹ Other applications are likely in oncology,³⁰ cardiology, and local blood-brain barrier function.^{31,32}

PET is a general technique which may be applied to any part of the body. Apart from metabolic and transmitter studies it may be used to measure tissue concentrations of any labelled substance or drug and its rate of change with time.³³ Nevertheless, its clinical utility is frequently questioned. Certainly, the technique is difficult (because of the short lived isotopes), expensive on capital costs, (necessitating a cyclotron, PET scanner, hot cells, and computing facilities), and