Lateral reading 10

METHODS AND SUCCESS OF NUCLEAR TRANSPLANTATION IN MAMMALS (McLaren A. Nature 1984;309:671–2)

Anne McLaren has reviewed this complicated subject and there could be implications in man. Thus, nuclear transplantation from couples at risk of transmitting a genetic defect to their progeny could provide a method of screening that did not involve termination of the (possibly) affected pregnancy. For example, if several eggs were removed from a woman and reinserted with inner cell mass nuclei from one of her own embryos, a few could be frozen while the genetically identical remainder would be screened for the genetic defect by chromosome or DNA analysis. In this way it would be possible to assure the woman that an embryo transferred back to her uterus from the frozen stock would be free from the screened-for genetic defect.

Agreed that the embryo replaced into the ‘at risk’ woman is normal, yet the previous manipulations seem far from trivial.


These two leaders appeared consecutively in the British Medical Journal of Saturday 18 August 1984. Both are good summaries of the two conditions but will not contain much that is new to clinical geneticists. In the brittle bone syndrome “we are seeing the thalassaemia of collagen” but when (yet again!) will someone look at Dupuytren’s contracture? In familial hypercholesterolaemia the point that was new to me is that there has now been formed a new self-help organisation, the Familial Hypercholesterolaemia Association. Its address is PO Box 612, London W2 2EE.

HOW DOES BONE MARROW TRANSPLANTATION CURE LEUKAEMIA? (Gale RP, Champlin RE. Lancet 7 July 1984:28)

Paediatricians and others have become accustomed to the idea of giving a marrow transplant to patients with acute myelogenous leukaemia—a heroic measure preceded by high doses of drugs and radiation—and it has always seemed a gift from Heaven when there is an identical twin to act as a donor.

In the present paper relapse rates in 31 transplants from monozygotic twins were compared with those in 339 transplants from HLA identical sibs managed with similar radiotherapy. Surprisingly, the relapse rate in the twins was 55% compared with 18±4% in the non-twin sibs. Various explanations for the differences are put forward but much the most interesting is that in those patients treated with allogeneic bone marrow there is a graft-versus-host reaction which gets rid of the remaining leukaemic cells.

The sibs need to be HLA compatible in order for the graft to take, but other incompatibilities seem to be favourable, though I wondered about the ABO groups which are not mentioned.


John Maddox writes “The geometrical regularity of living things has always been a source of wonder, some of it frankly superstitious. The bilateral symmetry of leaves, the axial symmetry of many flowers and the complex geometrical regularity of insect eyes are among the common observations of the natural world which, over the centuries, have excited speculation about the nature of the invisible hand that guides the development of living things”—and the solution may now not be far off via (as usual) Drosophila. The two relevant papers are summarised by Gary Struhl, starting with the observation that essentially all organisms are composed of an orderly series of parts. Blocks of genes are responsible for this homoeosis but occasionally mutants occur which result in the substitution of one or more segments by segments normally found elsewhere along the body axis. For example, legs may replace antennae on the head of a Drosophila which has had a mutation in the cluster of genes controlling antennae and legs.
More importantly it has been found that the DNA sequences in the homoeotic genes of Drosophila are similar to some which are found in the genomes of worms, frogs, mice, and man, the function of which has hitherto been unknown.

John Maddox concludes: "In Britain, the Warnock committee is about to recommend to the British Department of Health rules for the use of early human embryos in research, with a time-limit (said to be 15 days from fertilisation) for the duration of such work. Hitherto, embryologists have been hard pressed to suggest what observations might usefully be carried out. That is no longer the case".

IN SEARCH OF THE GENE (van Heyningen V. Nature 1984;311:104–5)

The 9th International Cystic Fibrosis Congress was held in Brighton on 9–15 June 1984. The enigma of the primary gene defect still eludes the experts but there was a good review of the conference in Nature, 13 September 1984. This gives valuable references and a large section of it is quoted verbatim.

"Despite the steadily improving prospects for afflicted individuals, the social implications of the disease are still serious, as was reflected in a study of parental attitudes in Californian cystic fibrosis families (M Kaback et al, University of California, Los Angeles). In 65 per cent of the 183 families studied, the affected child was the last born to the couple and more than a quarter of the couples resorted to surgical sterilization to avoid the possibility of having further offspring with the disease. Reliable prenatal diagnosis would clearly be welcome to such families.

Brock (Lancet 1983;i:941) and colleagues have found reduced levels of intestinal microvillar enzymes in amniotic fluid if an affected fetus is being carried in a pregnancy 'at risk' (previous cystic fibrosis child born to the parents). The reduced levels are thought to be secondary to the impaired passage of meconium in the cystic fibrosis fetus. Following the failure of an earlier prospective trial for reduced amniotic fluid protease activity (Nadler HL, Walsh MMJ. Pediatrics 1980;66:690) great caution is being exercised in confirming and extending these findings. Nonetheless, groups in Rotterdam (Kleijer WJ, et al, Erasmus University), Paris (Boué A, et al, Inserm U73) and Glasgow (Aitken DA, et al, University of Glasgow) are successfully applying microvillar enzyme estimations prospectively. Validation of this approach to prenatal diagnosis would be speeded up if definitive diagnosis of cystic fibrosis were possible in the fetuses declared abnormal and aborted on parental request. Confirmation of normal outcome in pregnancies carried to term is also difficult because there is considerable variability in severity and age of onset of overt disease, although neonatal sweat testing and immune-reactive trypsin measurement of newborns may help.

While there is every indication that prenatal diagnosis of this kind will help prevent the birth of a second affected child in families at risk, the assays are based on what is almost certainly a secondary phenomenon and the race to identify the basic gene defect is still on. Considerable excitement has been generated by recent work on isolated sweat glands (Quinton PM, Nature 1983;301:421) and on nasal epithelium (Knowles MR, Boucher RC, et al, University of North Carolina). In both cases, there appears to be a defect in ion transport in affected individuals. Thus, raised potential differences across the apical membrane of epithelial cells suggest reduced chloride permeability, and this is accompanied by reduced responsiveness to β-adrenergic stimulus by isoproterenol. The possibility that the abnormal β-adrenergic responsiveness and secretion in cystic fibrosis results from a defective calcium-dependent regulatory protein was suggested by several workers, for a variety of tissues (Case RM, University of Manchester; Cabrini G, et al, Centro Fibrosi Cistica, Verona; McPherson M, et al, University of Cardiff). One of the few groups to study heterozygote as well as homozygote red cells observes a calcium transport defect in both (Seymour CA, Davis JA, University of Cambridge).

As long as the basic gene defect remains unidentified, the choice of tissue for study will be arbitrary and often made on the basis of availability. The ability to culture sweat gland epithelia (Pedersen PS, Rigshospitale, Copenhagen) may prove a useful stimulus in this field. Meanwhile, considerable effort is being made to set up reliable heterozygote detection using serum samples (Manson JC, Brock DJH. Lancet 1980;i:330) as this would help in the search for the defective metabolic pathway and ultimately the primary mutant gene locus (Jamieson A, et al, University of Glasgow; van Heyningen V, et al, MRC Clinical and Population Cyto genetics Unit, Edinburgh; Guy-Cotte O, et al, Inserm U31, Marseille). Various teams (Williamson R, et al, St Mary's Hospital Medical School, London; Klinger KW, Case Western Reserve University, Cleveland; Lap-Chee Tsui et al, Hospital for Sick Children, Toronto; and Schwartz RH, et al, University of Rochester) are setting up DNA banks or permanent cell lines from cystic fibrosis families with two or more affected children.

The only available method of searching for the cystic fibrosis gene is linkage analysis by exclusion mapping using polymorphic DNA probes that have
been mapped to a known chromosomé region. In this way, using samples only from affected individuals and obligate heterozygotes, a number of chromosome regions have been found not to be linked to cystic fibrosis. Just before the congress took place, there was excitement about the possibility of assigning the cystic fibrosis gene to the tip of the long arm of chromosome 13 on the basis of an informative family with a segregating translocation (Edwards JH, Jonasson JA, Black NL. Lancet 1984;i:1020) but this now seems unlikely. Nonetheless, an air of optimism pervaded the conference because the introduction of new methods such as electrophysiology and gene cloning brings some promise of breakthrough."


"An infectious retrovirus vector has been used to transfer a bacterial gene encoding resistance to the neomycin analogue G418 into pluripotent haematopoietic stem cells present in explanted murine bone marrow tissue. Subsequent transplantation of the cells into lethally irradiated mice results in engraftment of the animals with donor haematopoietic tissue containing the bacterial gene. This approach affords an efficient and rapid means of re-introducing genetically modified tissue into intact organisms and provides a system whereby the expression and regulation of cloned genes can be followed within the context of a well characterized developmental programme."

I found the techniques described in this paper very difficult to understand and the possible applications of the research not clearly set out.

Fortunately David Weatherall has put the matter right for me and his very clear leader is therefore reproduced in full. It is now obvious to me that Williams et al have carried out work the principles of which are likely to be of importance in clinical medicine.

"The last few years have seen spectacular advances in our understanding of the molecular pathology of some human gene disorders. It turns out that the commonest and most extensively studied of these conditions, the thalassaemias, in which there is defective synthesis of the polypeptide chains of haemoglobin, result from a series of diverse structural mutations of the globin genes. But while the work on the thalassaemias has told us a great deal about abnormal gene action and has led to the development of methods for prenatal diagnosis by direct DNA analysis, it has not resulted in any improvement in patient management. Not surprisingly therefore, thoughts have turned to the possibility of replacing the defective globin genes. Successful treatment of thalassaemias in this way represents a formidable task; nonetheless, a paper on page 476 of this issue of *Nature* makes a step in the right direction.

Before it will be possible to insert globin genes into blood cells with any reasonable expectation of correcting a thalassaemia mutation, a number of difficulties will have to be overcome. The recognizable red-cell precursors in the bone marrow are already terminally differentiated, that is they are programmed to go through several divisions and then to become mature red cells destined to be destroyed after about 120 days in the circulation. Hence the target for gene insertion has to be a haematopoietic stem cell, a self-sustaining population from which all the blood cells (including white blood cells and platelets) are derived. These cells constitute less than 0-1 per cent of the marrow cells, cannot be isolated as a pure population and can be assayed only in murine systems which are not applicable to human experimentation. Furthermore, even if it were possible to introduce genes into a proportion of stem cells there is no reason why these cells should proliferate preferentially; the defect in haematopoiesis caused by defective globin chain production is only apparent when the globin genes are activated in the terminally differentiated erythroid precursors. Moreover, there is no guarantee that the inserted genes would be expressed only in red cells and not in white cells or platelets. Even if they were expressed in the appropriate cells their activity might not be synchronized with that of genes for other globin chains. For example, if β globin genes were inserted to cure β thalassaemia, it would be of little value if β chains were produced more rapidly than their partner α chains; the conversion of β thalassaemia to α thalassaemia would hardly be a tour de force for gene therapy.

Hitherto, these difficulties have been compounded by the fact that techniques for inserting foreign DNA into cells, using calcium microprecipitates for example, are extremely inefficient, achieving a transfection rate of approximately 1 cell in 10^3. Thus, using the most optimistic calculations, we might need 10^6 marrow cells—about 10–20 ml of human marrow—to have a chance of inserting a new gene into 1 stem cell. It was against this unpromising background that a few years ago an attempt was made to insert a normal β globin gene into the marrow cells of a patient with β thalassaemia. Given the difficulties involved, it is not surprising that this premature experiment failed, although at least the effect of the bad press that followed was to clarify
the criteria that would have to be met in any future attempts of this kind.

The paper by Williams et al in this issue of *Nature* suggests that at least one of the major obstacles to introducing foreign genes into marrow cells may have been overcome. Their elegant experiment entails inserting a marker gene into murine haematopoietic cells using a retrovirus vector and then following its fate using a well defined assay system for stem cells. If mice are irradiated at an appropriate dose they die of marrow aplasia. However, if marrow is injected after irradiation the animals can be rescued. The donor marrow populates the spleen in the form of discrete haematopoietic colonies which have been shown to be the progeny of single cells. The latter, which thus have the properties of stem cells, are called colony forming units—spleen (CFU-S). The Boston group constructed a defective retrovirus vector containing a neomycin resistance gene (NEO) from Tn5 and a mouse cDNA sequence encoding dihydrofolate reductase. They then transfected mouse marrow cells, injected them into irradiated mice and examined DNA prepared from spleen colonies by Southern blotting using a probe for the NEO sequence. About 10–25 per cent of the haematopoietic cells from the regenerating spleens contained the NEO-hybridizing fragment, suggesting that the recombinant genome had been transferred intact into a significant proportion of CFU-S. When CFU-S from these mice were transferred to a second group of irradiated mice, haematopoietic stem cells containing the fragment appeared in the spleens of the recipients, indicating that the transfected CFU-S had the capacity for self-renewal. Preliminary analyses suggest a single site of integration per CFU-S.

These results are extremely encouraging. Together with the recent work of Joyner et al who demonstrated the value of a similar but independently derived vector for gene transfer into mouse haematopoietic cells *in vitro*, they suggest that defective retrovirus vectors are able to achieve a very efficient level of integration into the DNA of haematopoietic stem cells, and that the transfected cells retain their capacity for self renewal. As the authors are careful to point out, many difficulties remain before this approach can be used for gene therapy. For example, nothing is known about the capacity for expression or regulation of genes inserted in this way, or about their structural integrity after multiple cell divisions. The safety and long-term stability of retrovirus-transformed cell populations will have to be explored. The present experiments were carried out in irradiated animals; it remains to be seen whether a system can be developed to promote preferential survival and proliferation of transfected stem cells in intact animals. Finally, up to 15 pg of globin may be needed per cell to correct a thalassaemia defect, so an inserted gene would have to be very efficiently expressed. Perhaps the most immediate application of the new approach will be for correcting enzyme deficiencies, where a much lower degree of expression of an inserted gene might be sufficient.

It is still early days, but the description of an efficient method for transfecting haematopoietic stem cells offers major encouragement to those who believe that the ultimate goal of clinical genetics is gene replacement therapy, rather than termination of pregnancy."


"Two patients with severe neurological symptoms were given zinc sulphate by mouth three times daily in doses of 200 mg, later increased to 300 mg. One patient, a 21 year old man, started to receive zinc sulphate after his condition had deteriorated during treatment with cupriuretic drugs. The other, a 27 year old woman, was treated from the start with zinc sulphate. The conditions of both patients improved appreciably, and they were still receiving treatment with zinc sulphate roughly two years later. Effective depletion of body copper stores was shown by an intravenous radiocopper loading test and liver biopsy. No side effects were found.

Wilson's disease may effectively be treated with zinc sulphate alone."

**Abstracts—are they really a dud?**

"Very few practising scientists doubt the value of the well prepared abstract as it often provides essential information. Nevertheless, as your recent editorial points out, many abstracts are not well prepared and can be misleading. These defects, however, are no monopoly of the abstract and are to be found equally in many full papers, particularly in journals which do not apply a system of rigorous independent review. Nevertheless, anyone with more than a passing acquaintance with leading biomedical journals must know how widely the abstract is used as a vehicle for disseminating scientific data.

It would be a pity if the quick, concise form of transmitting information provided by abstract publication were to be done away with. For those who attend the meeting where the work is presented the abstract provides a useful aide memoire, and for
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those unable to attend a means of learning what went on. All over the world there is an avid readership for abstracts among those who wish to know as soon as possible what is going on elsewhere. There are others, of course, who have no particular wish for this information—the editor of the Archives may be one—and for them obviously the abstract has nothing to offer.

The problem is how to monitor abstracts in order to suppress those which have been carelessly prepared while encouraging publication of those which seem to provide a good account of what was found. Two methods seem to be available. One, used by societies on both sides of the Atlantic, is to leave selection to a committee, in which case the process is inevitably secret and oligarchic. The other is for those present at the meeting, having had the opportunity both to read the abstract and to hear the paper, to vote on its suitability for publication. They can also use this opportunity to point out discrepancies or insist on corrections and insertions as a condition of publication. Although this method of vetting abstracts is far from perfect, it is open, democratic, and carries the advantage of stimulating healthy debate. In my opinion, it is much the best of all editorial devices available for judging an abstract. To criticise the voting system because it might cause embarrassment is to raise an objection which could destroy any democratic means of decision making.

Many readers and writers of abstracts will no doubt tremble to learn that they are to be totally abjured by the Archives. Even references to abstracts are to be forbidden. Nevertheless, it seems likely that the abstract habit will continue, even if the whole United Kingdom becomes an area of total abstinence—a probable event, however, only within the narrow confines of paediatrics. Surely we should aim not to suppress abstract publication but to encourage, in the various societies to which we belong, the habit of proper monitoring and criticism of abstracts. A meeting is probably not worth attending if no steps are taken to guarantee the scientific and ethical standards of the work presented and to ensure that this is properly reflected in any associated publications. To say that many meetings and abstracts fail to meet these standards is only to repeat what we already know very well—that in all branches of medicine many meetings and many publications are of an unsatisfactory standard. I submit that this situation is unlikely to be remedied by abolishing the abstract, which has just as much chance of being good or bad as any other form of publication."

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