Green College Lectures

Molecular biology at the bedside

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By the 1960s the dust was settling after a period of great excitement in the biological sciences. It was already established that DNA is a self replicating double helix and that the hereditary message of genes is contained in triplets of nucleotides. It was also known that DNA acts as a template for the production of messenger RNA, which is transported from the nucleus to the cytoplasm to act as a blueprint for protein synthesis, and that amino acids are brought to the messenger RNA template on transfer RNA molecules. And a picture was emerging of how ribosomes interact with these different types of RNA to put together a peptide chain. But these remarkable discoveries had little impact on the clinical world, which found it easier to accept the conclusions of authors such as MacFarlane Burnet, who in his book *Dreams, Genes, and Realities* wrote: "I cannot avoid the conclusion that we have reached the stage in 1971 when little further advance can be expected from laboratory science in the handling of the intrinsic types of disability and disease."¹

In the 15 years since Burnet and others wrote off the usefulness of the basic biological sciences there has been a second revolution in biology, which, though perhaps more technical than conceptual, has completely changed the picture. Methods have been developed for cutting up genes, cloning them in bacterial cells, and isolating them in bulk for structural or functional analysis. In a remarkably short time the stage has been reached when almost any gene worth studying can be isolated and its fine structure determined. But the medical sciences have again been slow off the mark, particularly in Britain. As recently as 1984 Peter Newmark wrote in an editorial for *Nature*: "for too many years those that felt obliged to respond to the taunt that molecular biology, for all the medical research money it had soaked up, had never done anything to help diagnostic medicine, could, at best, fall back on the case of the thalassaemias."² Indeed, it is only in the past year or two that clinical scientists have started to see the extraordinary potential of what molecular biology has on offer.

Since, as pointed out by Newmark, the haemoglobin disorders, particularly the thalassaemias, have played such a central part in taking molecular biology to the bedside, it will repay a brief examination of how this branch of research has developed and of the subtle interaction between the basic and clinical sciences which has allowed us to reach the stage at which recombinant DNA technology can be applied directly in medical practice.

The first "molecular disease"

Human biochemical genetics was born in 1901, when Archibald Garrod first related a biochemical abnormality to an inherited disease. But it was not until 1949 that Linus Pauling and his

colleagues first discovered the molecular basis for a genetic disease. During an overnight train journey after a committee meeting in Denver in 1945 Pauling fell into a conversation with William Castle of Boston, who told him that when sickle cells are deoxygenated they show birefringence in polarised light. Realising the significance of this observation, Pauling asked one of his research students, Harvey Itano, to investigate the haemoglobin of patients with sickle cell anaemia. Using a modified Tiselius moving boundary electrophoresis apparatus, they found that the haemoglobin from such patients has a different mobility to that of normal haemoglobin. This told them that the globin structures must differ, an observation which led Pauling and Itano to use the term "molecular disease" to describe sickle cell anaemia.

The work of Pauling's group could not have come at a more opportune time. In Cambridge Sanger was developing his ingenious techniques for determining protein structure and Perutz was engaged in the painstaking crystallographic analysis of haemoglobin. In this stimulating environment a young research worker, Vernon Ingram, decided to apply Sanger's methods to study the structure of sickle cell haemoglobin; by a fortunate chance some samples had been left in Cambridge by Anthony Allison, who with Perutz had been attempting to crystallise sickle cell haemoglobin. Ingram digested the haemoglobin with trypsin. Although one dimensional separation of the tryptic peptides showed no difference from those of normal haemoglobin, when they were separated in two dimensions by electrophoresis and chromatography the resulting "fingerprints" showed a clear difference in the position of one tryptic peptide. Ingram went on to sequence the aberrant peptide and found that haemoglobin S differs from normal haemoglobin by the substitution of a single amino acid, valine for glutamic acid.

Meanwhile, protein chemists in Germany and the United States were showing that human haemoglobin consists of two pairs of peptide chains, which they called α and β chains; Ingram went on to show that the amino acid substitution in sickle haemoglobin is in the β chain. In 1959 Smith and Torbert in Baltimore discovered a large family in which two abnormal haemoglobins were present, haemoglobin S and a hitherto undescribed variant which segregated independently from haemoglobin S. The "new" haemoglobin, which was christened Hopkins 2, was found to have an abnormal α chain. The position was now clear. Human haemoglobin consists of a pair of α chains and a pair of β chains, which must be under the control of distinct α and β globin genes. The one gene one enzyme model of Beadle and Tatum, based on their classic studies of *Neurospora*, could now be further refined; a "gene" is a unit which directs the structure of a polypeptide chain. The following years were to see innumerable examples of haemoglobin variants and, with the final solution of the structure of human haemoglobin by x ray crystallography, the development of an elegant picture of how the protein works as an oxygen carrier.

Within a year or two a more complete picture of the genetic control of the human haemoglobins emerged. Walter Schroeder discovered that fetal haemoglobin (Hb F) also has α chains, which are identical to those of adult haemoglobin (Hb A), whereas its partner chains differ; Schroeder called the latter γ chains. At about this time Henry Kunkel, one of the great names of American immunology, made a brief foray into haemoglobin studies and discovered that normal adults have a minor haemoglobin called haemoglobin A₂; a little later this was found to also have α chains, in this case combined with another set of structurally distinct chains called δ chains. Thus by the early 1960s a good working model of the genetic control of human haemoglobin had been established. Alpha chain genes are active in fetal and adult life; in the fetus α chains

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combine with γ chains to make haemoglobin F ($\alpha_2\gamma_2$) while in adults they combine with β and δ chains to make haemoglobins A ($\alpha_2\beta_2$) and A₂ ($\alpha_2\delta_2$).

Thalassaemia as a haemoglobin disorder

A severe form of anaemia leading to death within the first few years of life was first described by a paediatrician from Detroit called Thomas B Cooley. Because all the early cases encountered in the USA came from the Mediterranean region George Whipple and his colleagues decided to call the condition thalassaemia ($\theta\alpha\lambda\sigma\sigma\alpha$), a name which, although it subsequently turned out to be inappropriate, has stuck. The carrier state for this condition was first recognised at about the same time in Italy, but because of language difficulties and problems of scientific communication during the period leading up to the second world war the genetics of thalassaemia were fully established only during the late 1940s. Over the next decade it became apparent that this condition is not restricted to the Mediterranean region but has a worldwide distribution.

After the description of sickle cell haemoglobin by Pauling and Itano it is not surprising that in the period after 1950 the haemoglobin of patients with thalassaemia came under close scrutiny. Several novel and at first apparently unconnected observations were made. For example, it was found that many children with severe thalassaemia have persistent Hb F production; normal infants lose their fetal haemoglobin by the end of the first year of life. Kunkel noted that the carrier parents of severely affected thalassaemic children sometimes but not always have raised concentrations of haemoglobin A₂. And it was found that some patients with the clinical picture of thalassaemia have an abnormal haemoglobin consisting of four β chains (β_4) with no α chains. This variant was called haemoglobin H—the next available letter of the alphabet available for a new haemoglobin in 1955. To complete this puzzling picture, in 1959 a haemoglobin which consists of four γ chains (γ_4) was discovered by Herman Lehmann when he was studying the blood of a baby with thalassaemia at St Bartholomew's Hospital, London. Since by now there were no letters of the alphabet left with which to name new haemoglobins he called this novel fetal haemoglobin variant "Bart's."

Another critical observation for the development of ideas about the molecular basis of thalassaemia came from the study of patients who had inherited a sickle cell gene from one parent and thalassaemia from the other. This condition, sickle cell thalassaemia, was first discovered by Silvestroni and Bianco in Italy in 1945 but their observation was largely ignored until the advent of haemoglobin electrophoresis in the mid 1950s. Pauling and Itano had found that sickle cell carriers have more haemoglobin A than S. It turned out that many patients with sickle cell thalassaemia have the reverse, more haemoglobin S than A. In other words, the sickle cell and thalassaemia genes interact with each other in a highly specific way. Furthermore, children of patients with sickle cell thalassaemia have either sickle cell trait or thalassaemia trait; they are never normal or doubly affected. This suggested that the thalassaemia and sickle cell genes are alleles. Nevertheless, a few exceptional families started to crop up in whom it was clear that the thalassaemia "gene" was not an allele of the sickle cell gene.

In 1959 Ingram and Stretton, in a theoretical paper which had a major influence on the subject, attempted to synthesise these observations. They suggested that there are two types of thalassaemia, α and β . Defective β chain synthesis would explain the raised level of γ (haemoglobin F) and δ (haemoglobin A₂) production in many cases of thalassaemia. On the other hand, if there is defective α chain synthesis (α thalassaemia) the output of haemoglobins A, F, and A₂ would all be affected, since they all have α chains. But a paucity of α chains would lead to the production of excess γ or β chains and hence would explain the association of β_4 and γ_4 tetramers with some types of thalassaemia. And, most important, this model explained the interaction and allelism of some forms of β thalassaemia with the sickle cell gene. If it is assumed that the sickle cell mutation somehow reduces the output of β^S chains—the structure-rate hypothesis developed by Pauling and Itano—a sickle cell carrier with a normal (β^A) and abnormal (β^S) gene should make more haemoglobin A ($\alpha_2\beta_2$) than S ($\alpha_2\beta_2^S$). On the other hand, an individual who inherited a β thalassaemia gene (β^T) from one parent, and a sickle cell gene (β^S) from the other, would have the genotype $\beta^T\beta^S$; since the action of the β thalassaemia gene is to reduce the output of normal β chains, they would have more haemoglobin S than A. Furthermore, a person with the genotype $\beta^T\beta^S$ could not have normal children; their offspring must either have the thalassaemia (β^T) or sickle cell (β^S) genes. It also followed that an individual with α thalassaemia and the sickle cell trait would not show a reversed ratio of haemoglobin A and S: α chain deficiency should affect the production of haemoglobins A and S equally. And a family study would

show independent segregation of the conditions: α and β chains are controlled by separate gene loci and hence α thalassaemia and sickle cell haemoglobin (a β globin mutation) cannot behave as alleles.

Here then was a group of genetic disorders characterised by a reduced rate of production of one or more peptide chains of a protein. Furthermore, it was already clear that these disorders are clinically and genetically heterogeneous and that "thalassaemia" must represent a family of disorders of the rate of globin chain production. The stage should have been set for rapid progress towards understanding the molecular basis of diseases caused by a reduced rate of protein synthesis. Nevertheless, it was another 15 years before it was possible to define unequivocally the molecular defect in even one form of thalassaemia.

The quiet years, 1960-75

After the excitement of the discovery of the molecular basis for sickle cell disease and the elucidation of the genetic control of human haemoglobin the topic suddenly went quiet. Many of the protein chemists whose work had led to these discoveries gave up haemoglobin research and moved to other pastures. For, although it was clear that the thalassaemias offered an exciting prospect for understanding human genetic disease, nobody had any idea where to go next.

In an extension of Pauling and Itano's structure rate hypothesis Ingram and Stretton suggested that defective globin chain synthesis in the thalassaemias might be due to silent amino acid substitutions—that is, replacements that do not alter the charge of the haemoglobin molecule but which somehow lead to a reduced rate of synthesis. This idea was shown to be incorrect, at least for one type of β thalassaemia, when the β chains were sequenced and found to be normal. It was also suggested that at least some forms of thalassaemia might be due to mutations at regulatory loci which do not concern the structure of the genes. Models, derived from the elegant work of Jacob and Monod on the control of gene expression in microorganisms, proposed that the thalassaemias might result from mutations at putative operator or regulator loci. But it always seemed likely that the control of protein synthesis in human beings might be considerably more complex than in *Escherichia coli* and, furthermore, none of these models was amenable to experimental testing.

Nevertheless, a few clinical groups, undeterred by these difficulties and realising the potential of these disorders for understanding the molecular pathology of disease, decided to press ahead and study in vitro haemoglobin synthesis in thalassaemic cells. This approach was based on earlier studies by Shemin, Borsook, and others, who had shown that it was possible to label both haem and globin in short term culture of rabbit reticulocytes. Marks and his colleagues showed that haemoglobin A synthesis is defective in β thalassaemia and that ribosomal function is normal in thalassaemic cells, and Heywood and his colleagues showed unequal labelling of α and β chains of haemoglobin in thalassaemic red cells although the methods that they were able to use were, at best, semi-quantitative. Nevertheless, in 1965 John Clegg, Michael Naughton, and I worked out a way of separating the haemoglobin chains in a way which yielded almost 100% of the starting material. Thus we were able to label reticulocytes with radioactive amino acids and to measure accurately the total amount of α and β chains synthesised during the period of study. Over the next two or three years we and others used this technique to analyse many different types of thalassaemia. These in vitro studies showed unequivocally that the α and β thalassaemias are due to imbalanced synthesis of globin chains. But we were still no nearer to knowing why there is defective production of α or β chains.

We then spent several years attempting to apply the techniques developed by Howard Dintzis for studying the assembly of rabbit globin genes to the thalassaemia problem. By 1967, after numerous setbacks and failures, we could convince ourselves that the rates and patterns of assembly of β chains are the same in thalassaemic and normal reticulocytes. This suggested that the β thalassaemias must result from a reduced output of β globin chain messenger RNA; such messenger RNA that is made is functionally normal. In other words, the translation of β globin messenger RNA appeared to proceed in a normal way from the 5' to the 3' end, thus ruling out a major defect in translation or termination. These results were subsequently confirmed when it became possible to assay human messenger RNA in cell free systems.

The advances that facilitated the further molecular analysis of thalassaemia came from two unexpected and totally unrelated disciplines. In 1964 Temin predicted that enzymes must exist for synthesising DNA on RNA templates. The enzyme—reverse transcriptase, to give it its more palatable name—was isolated independently by Temin and Mizutani and by Balti-

more in 1970. It was now possible to make DNA copies from specific messenger RNA templates; with pure α or β chain messenger RNA templates complementary DNA (cDNA _{α} or cDNA _{β}) copies could be synthesised. Furthermore, if radioactive nucleotides were incorporated into the reaction these cDNAs could be labelled. The other major advance was the development of molecular hybridisation. If DNA or double stranded RNA is heated the two strands come apart and re-anneal on cooling. These re-annealing reactions are highly specific and quantitative. If after denaturation radioactively labelled cDNA is added to the mixture of re-annealing DNA, or to RNA, because of the rules of base pairing, it will bind only to the DNA or RNA if it finds identical base sequences. Thus radioactive cDNA can be used as a sensitive "probe" to examine DNA or messenger RNA for the presence of these sequences. Hence it was possible to use cDNA/RNA hybridisation to confirm that there is a reduced amount of hybridisable β globin messenger RNA in the cells of patients with β thalassaemia, and vice versa in α thalassaemia.

A question of particular interest to molecular biologists in the 1960s was the number of copies of a structural gene for a protein such as globin in the total genome. This problem was solved by Bishop and others using molecular hybridisation techniques with a vast excess of cDNA under very stringent conditions; it turned out that there are only a few copies of the globin genes. It occurred to us that if this method could be used to estimate the number of copies of normal genes it might also tell us whether the globin genes were present or deleted in some forms of thalassaemia. In 1970 we had shown that stillborn babies homozygous for α thalassaemia make no α globin chains. We wondered, therefore, if their α globin genes had been deleted. Lacking the technical skill with which to carry out molecular hybridisation experiments, we approached John Paul in Glasgow and asked whether his group would be willing to collaborate in an experiment to see if these stillborn babies had lost their α globin genes. The experiment was put into the hands of a research fellow working with John Paul and Bob Williamson called Sergio Ottolenghi. In 1974 a clear result was obtained: these babies have a complete or almost complete deletion of their α globin genes. This experiment, which was reported independently at the same time by Y W Kan and his colleagues in the USA, was the first direct demonstration of a gene deletion in man and was the start of an extraordinary period of activity in thalassaemia, which by 1985 saw the complete solution of the molecular basis of the condition.

The last five years

To appreciate why the molecular basis of many forms of thalassaemia, and more recently other genetic diseases, has been worked out in such a short time, it is necessary to consider the remarkable developments in the technology of molecular biology which took place in the 1970s. Firstly, a family of enzymes called restriction enzymes were discovered which cut DNA at very precise sequences. Secondly, ingenious methods were developed for inserting pieces of DNA into plasmids or bacteriophage vectors and for growing these recombinants in bacterial cells. In this way it was possible to make "libraries" representing the entire human genome. Hybridisation techniques were evolved to isolate genes of interest from these libraries and rapid techniques for DNA sequencing were developed. Thus by the late 1970s all the tools required to analyse the fine structure of genes were available.

The painstaking work of several clinical groups over the previous 20 years had resulted in the human haemoglobin loci being the best characterised set of genes in man, or any other higher organism for that matter. Furthermore, it was already apparent that the thalassaemias were a family of well characterised genetic disorders of globin synthesis which were ideal for exploration by these new techniques. So, just as the protein chemists had descended on human haemoglobin in the 1950s, the molecular biologists moved in in the late 1970s.

Within two or three years all the human globin genes had been isolated and sequenced and their order in the globin gene clusters and much of their structure had been determined. At first there were no major surprises: the arrangement of the genes as predicted from simple family studies was correct. But about this time something of a molecular bombshell was dropped when it was discovered that most mammalian genes are discontinuous—that is, their coding regions are interspersed with non-coding

sequences called introns. It turned out that primary transcripts of these genes contain both introns and coding or exon sequences; the former have to be cut out and the exons spliced together in the nucleus before the processed messenger RNA is delivered to the cytoplasm. It was also found that all mammalian genes have conserved sequences at their 5' (upstream) ends, which are now known to be of great importance for the regulation of their rates of transcription. These observations opened up a whole new series of possibilities for defects in protein synthesis.

Thus, with the ability to clone and sequence human genes, and the availability of rapid techniques for studying their fine structure by gene mapping, all the tools were available for studying the thalassaemias at the molecular level. Over two or three years a remarkable story emerged. More than 60 different mutations were found in patients with thalassaemia. These included partial or complete deletions, point mutations in exons which lead to premature chain termination or scrambling of the genetic code, mutations in introns or exons which interfere with splicing of the messenger RNA precursor, inversions of whole regions of the genome, and, even more interestingly, point mutations outside the structural genes in the conserved "regulatory sequences." Thus in an incredibly short time we appeared to have a good idea of the entire repertoire of things that can go wrong with our genes.

Not surprisingly these remarkable advances soon spread outside thalassaemia. Several other human genetic diseases were known to result from defective production of specific enzymes or other proteins and it was not long before genes for phenylalanine hydroxylase, antithrombin III, and some of the blood clotting factors were isolated; molecular defects similar to those which cause thalassaemia were found. Indeed, any genetic disease due to a deficiency of a known enzyme is now amenable to this approach.

But this new found ability to analyse the human genome has had even more far reaching consequences for human genetics. Unlike the haemoglobin disorders the cause of many genetic diseases and the chromosomal location of the genes involved are completely unknown. Geneticists had realised for many years that one approach to this problem is to find a gene whose product can be identified and to carry out family studies to see if a linkage can be established between the "marker" gene and the gene that is being sought. Nevertheless, very few marker genes of this type were available and for a long time this approach had very little practical value. But the new techniques of DNA analysis have changed all this. Every few hundred bases or so throughout the human genome there are single base changes which either produce new restriction enzyme sites or remove previously existing ones; these harmless polymorphisms can be used as linkage markers to produce a map of the human genome.

With this approach it has been possible to find close linkages for disorders of unknown aetiology such as Huntington's chorea, muscular dystrophy, polycystic kidney disease, and, most recently, cystic fibrosis. With a linkage established it should be possible, by various tricks of recombinant DNA technology, gradually to move closer to the gene and ultimately to define and sequence it. In this way it is hoped that it may be possible to "work back" to the protein sequence and hence to determine the exact cause of some of these conditions. (No doubt workers in the subject will sleep more easily when such a tour de force has been achieved, even once.)

These advances have had important practical implications for diagnostic genetics. With either direct gene or linkage analysis it is now possible to identify many single gene disorders in DNA obtained by chorion villus sampling or amniocentesis and this approach is already being used widely for the detection of carriers and prenatal diagnosis. And, of course, recombinant DNA technology is having direct clinical applications in many disciplines outside genetics. A variety of important pharmaceutical agents and blood products have been produced by genetic engineering. The characterisation of the remarkable rearrangements which occur in the immunoglobulin genes during development has provided a valuable set of markers for typing different forms of leukaemia and lymphoma, and the discovery of cellular oncogenes is providing valuable new insights into the mechanisms of neoplasia. And a start has been made in dissecting the complex interactions of genotype and environment which underlie many of the common killing diseases of Western societies. Indeed, it is now clear that the next few years will see a major change in emphasis in medical research from the "whole patient" to the cellular and molecular pathology of disease processes.

Relations between basic sciences and clinical research

Does this remarkable story tell us anything about the interaction between the basic and clinical sciences? At a symposium on molecular haematology at the 1968 meeting of the British Society for Haematology in Cambridge I presented the thalassaemia story as it then was, and summarised some of our conceptual and practical difficulties in advancing much further. In summing up Sidney Brenner suggested that our problems were relatively trivial and could be solved within a few months by molecular biologists. Though thoroughly chastened at the time, I wonder in retrospect whether this was really the case.

As I pointed out earlier, the basic scientists descended on human haemoglobin in two short waves, both at times when there were problems to be solved which were well defined, of general biological interest, and amenable to newly developed techniques. Thus in the 1950s and early 1960s protein chemists needed no prompting to study the structure and structure/function relationships of human haemoglobin; along the way, and because William Castle asked the right question, they discovered the structural haemoglobin variants which led to an understanding of the genetic control of human haemoglobin. Much later—and after many years of painstaking work in clinical departments which provided a clear picture of the likely arrangement of the globin genes and the enormous potential of the thalassaemias as models for studying their regulation in higher organisms, and when the new methods of recombinant DNA technology had been developed—there was a second period of intense interest, this time on the part of molecular biologists. Over a few years they worked out the structure and arrangement of the globin genes and took a short lived but highly productive interest in their molecular pathology.

This may be the future pattern for the development of human molecular biology and molecular medicine. There will be exceptions, like the remarkable story of Goldstein and Brown's years of work on the low density lipoprotein receptor and cholesterol metabolism. But by and large the non-clinical scientist will always want to tackle questions of broader biological interest with well defined experimental systems which yield clean answers. They must be supported and allowed to follow their noses in this way; none of the remarkable developments in recombinant DNA over the past 15 years originated in work which started out with an applied aim in mind, and yet these advances have opened up vast areas of direct relevance to clinical medicine.

I suspect that the next phase of development in human haemoglobin will follow the same pattern. The central question for those interested in clinical medicine is how the globin genes are regulated during development: if we knew how to manipulate the switch from fetal to adult haemoglobin we could ameliorate sickle cell anaemia and β thalassaemia. Yet virtually nothing is known about the developmental regulation of genes. Thus a great deal of work will have to be done on globin to try to formulate the appropriate questions and learn something about the regulatory regions of the globin genome and the types of molecules concerned. But I suspect that the real break will not come for some years and may well result

from totally unrelated work on the developmental regulation of gene expression in organisms such as *Drosophila*, where it is possible to ask much cleaner questions. But sooner or later the two topics will merge.

Clearly the basic sciences should be well supported and left alone, while the clinical world must learn how to make use of the extraordinary opportunities which cell and molecular biology have presented to it over the last few years. This will require a major reorganisation of clinical research; rather than separate specialties such as cardiology and endocrinology we shall have to develop multidisciplinary research units using the same general methods of cell biology and recombinant DNA technology to analyse disease at cellular and molecular levels. As things become more complex we shall need teams made up of both clinical and non-clinical scientists working together on these problems. And if the dual approach to human molecular biology, as outlined above, is to be effective we shall have to develop a new breed of clinical scientists who, like William Castle, can communicate with the basic scientists and ask the right questions at the right time.

Compared with the USA and other countries, the UK has been particularly slow to see the importance of molecular and cell biology for clinical research. As pointed out by Sir Christopher Booth in his recent Harveian lecture, the relationship between clinicians and basic scientists has never been very happy in Britain.³ Peter Medawar has also written on this topic, pointing out that this hiatus between pure and applied science is a curiously Anglo-Saxon attitude.⁴ In a medical setting I suspect that much of this stems from a lack of common purpose between preclinical and clinical teachers. Most young people who enter medical schools want to be doctors. To fire the imagination of those few who will go on to become our most talented clinical scientists we may need to rethink how the biological sciences are taught in our preclinical schools. Much of biochemistry, physiology, genetics, cell biology, and so on can now be presented in a more applied context without diluting its scientific respectability.

Our lack of success in basic clinical research over the past few years is usually ascribed to financial deprivation. Though undoubtedly this has not helped, I suspect that our problems go much deeper, reflecting as they do serious defects in undergraduate medical education compounded by a rigidity of thinking in postdoctoral training which is having a stultifying effect on many of our most talented graduates. If we are to take advantage of recent developments in the biological sciences, we may have to take a fresh look at some of our ossified attitudes to medical education at every level.

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Is there any evidence of the effectiveness of the methods published in women's magazines for conceiving a child of a particular sex?

Methods of choosing the sex of one's baby have been described throughout history and include douches, variations in coital position, and attention to the timing of orgasm.¹ None of these is effective, but recently more interest has been shown in the timing of intercourse relative to ovulation. A well publicised theory developed by Shettles suggests that the smaller, γ bearing sperm move more rapidly in the female genital tract but lose their fertilising capacity more quickly; therefore if insemination occurs close to the time of ovulation a male sperm is likely to reach the ovum first, whereas if intercourse takes place several days before ovulation the male sperm will become non-viable before the ovum is released.² A careful prospective study, however, entailing hormone estimations in 33 women concluded that if anything the trend was in the opposite direction.³

Another study of Shettles's technique, concerning 73 couples over four

years, showed no difference from the sex ratios resulting from uncontrolled intercourse.³ Because of disappointment with these methods, research on sex preselection is now focusing on the manipulation of semen to separate X and Y bearing sperm—for example, by density gradient centrifugation.⁴ Such laboratory methods may give a 75% chance of conceiving a child of a particular sex.¹ At present the only accurate method of choosing the baby's sex is prenatal diagnosis during the first or second trimester, followed by selective termination of pregnancy, and this is justified only in cases of severe sex linked disease.¹—JAMES OWEN DRIFF, senior lecturer in obstetrics and gynaecology, Leicester.

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