Parotid Tumours

The patient with the parotid swelling presents a classical diagnostic problem. If it is smooth, mobile, and long-standing no matter its size then a mixed salivary tumour (pleomorphic adenoma) is the most likely diagnosis, though in some cases the diagnosis will be adenolymphoma. Fixation to the skin or deeper structures, ulceration, pain, facial palsy, or ear discharge suggest malignant change in a pleomorphic adenoma or a frankly malignant tumour such as an adenocystic or mucoepidermoid carcinoma. One may go along the traditional diagnostic road of, for example, soft tissue x-rays examination or sialograms, but at the end of the day most patients will come to surgery. If the diagnosis is in doubt a frozen section at the time of surgery may help. In pleomorphic adenoma or adenolymphoma the superficial lobe is usually involved and a superficial parotidectomy with sparing of the facial nerve is curative. In malignant tumours a total parotidectomy with or without a neck dissection is indicated and a resulting facial palsy must be accepted. If overlying skin is involved this is widely excised and if there is bony invasion then a partial petrosectomy by a combined intracranial and extracranial approach is desirable. Radiotherapy is of little value in the treatment of these lesions.

In most pleomorphic adenomata with malignant change the

results of radical surgery are very good. Adenocystic and mucoepidermoid carcinomata have a definite tendency to local recurrence even years after resection. They also tend to metastasize to cervical lymph nodes and lung. Fortunately, patients may live symptomless for many years with pulmonary involvement, especially with adenocystic carcinoma.

Conclusion

Radical surgery has undoubtedly improved the prognosis in head and neck cancer, but what is the quality of life attained by the survivors? Life is sweet and, after a difficult period of adjustment, when medical advisers and relatives must do all they can to help, most patients settle down, many return to work, and almost all are grateful for being cured; psychological problems are minimal.

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Occasional Survey

Molecular Basis for some Disorders of Haemoglobin Synthesis*—II

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Thalassaemias

The thalassaemia syndromes are a heterogeneous group of disorders characterized by a reduced rate of synthesis of one or more of the globin chains of haemoglobin. These conditions are the commonest haemoglobinopathies. They occur sporadically in every racial group and present a massive public health problem in many parts of the world. In areas where these conditions are highly prevalent, with carrier rates of between 5% and 20%, the large numbers of homozygotes cause a major drain on blood transfusion resources.

HETEROGENEITY OF THALASSAEMIAS

Many different disorders of haemoglobin synthesis can produce the clinical picture of thalassaemia. These may be classified into two main groups, however, depending on which globin chain is produced ineffectively—that is, the α -thalassaemias and the β -thalassaemias.¹ Within each group there are many different varieties. Furthermore, the thalassaemia genes may be inherited

together with those for structural haemoglobin variants so as to produce a complex series of clinical disorders all characterized by a reduced rate of globin-chain synthesis. In Thailand, for example, over 50 such conditions have been identified.¹

CELLULAR PATHOLOGY OF THE THALASSAEMIAS

All the thalassaemia syndromes have in common a reduced rate of synthesis on one-half of the haemoglobin molecule and thus imbalanced globin-chain production.²⁻⁴ In the β -thalassaemias this leads to the production of an excess of α -chains in many of the red-cell precursors. Since fetal haemoglobin (γ -chain) synthesis persists in some cell lines part of the excess α -chain is combined with γ -chains to produce haemoglobin F. This occurs in only a limited number of red-cell precursors and others are left with a large number of α -chains with no partner chains. The uncombined α -chains are unstable and precipitate, producing large inclusion bodies.⁵ The presence of these inclusions causes considerable abnormalities of cellular maturation and proliferation and many of the affected red-cell precursors never leave the bone marrow. This is the mechanism for the striking degree of ineffective erythropoiesis common to all the thalassaemia disorders. Such cells as do leave the marrow have a shortened survival because they suffer damage due to the presence of rigid inclusions, and many of them are sequestered in the spleen and other parts of the reticuloendothelial system. Thus the anaemia of severe β -thalassaemia results from a combination of ineffective production and reduced survival of the red cells.

Most of the clinical findings of β -thalassaemia can be related to the primary imbalance of globin-chain synthesis.^{4, 6, 7} Affected

*Conclusion of the 1974 Watson Smith lecture given at the Royal College of Physicians of London on 8 May 1974.

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children are severely anaemic, and in addition many of the viable red cells contain relatively large amounts of haemoglobin F which has a high oxygen affinity. The severe anaemia and the fact that the red cells do not give up their oxygen as readily as those of normal adults combine to produce a gross degree of tissue anoxia which in turn leads to the production of large amounts of erythropoietin. This stimulates red-cell proliferation and massive expansion of the bone marrow. Indeed it has been estimated that the total erythroid tissue mass hypertrophies about twenty-fold in β -thalassaemia⁷ and it extends throughout the bone-marrow cavities and causes thinning of the bones and considerable skeletal deformities. It may also appear in a variety of extramedullary sites. This massive turnover of tissue causes several clinical features including a hypermetabolic state with wasting and fever, which in some cases simulates a malignant disorder, hyperuricaemia, and increased folate requirements. The erythroid hyperplasia also results in increased iron absorption and this, together with repeated blood transfusions, causes a massive degree of iron overload, and most affected children die of haemosiderosis, affecting particularly the myocardium, in the second decade.

The molecular pathophysiology of the α -thalassaemias follows a rather similar pattern with certain modifications. A deficiency of α -chains in intrauterine life or in infancy results in an excess of γ -chains while a similar deficiency in adult life causes an excess of β -chains. Free γ -chains or β -chains can aggregate to form viable molecules with the formulae γ^4 and β^4 , called Hb Bart's and Hb H respectively. These do not precipitate extensively in the bone marrow and hence the degree of ineffective erythropoiesis in the α -thalassaemias is less than in β -thalassaemia. Though cells containing Hbs Bart's and H escape into the circulation, however, these haemoglobins are unstable and precipitate, causing intracellular inclusion formation and haemolytic anaemia. As well as being anaemic patients carrying these haemoglobins are at a further disadvantage since, because Hbs Bart's and H have no α -chains, they lack the normal co-operative activities between unlike chains which result in a physiological oxygen dissociation curve and, in effect, do not give up their oxygen. Thus patients with α -thalassaemia in addition to having a haemolytic anaemia are further embarrassed by having variable amounts of useless haemoglobin in their red cells.

Molecular Defect in Thalassaemia Syndromes

Though we have a relatively clear picture of the pathophysiology and cellular pathology of the thalassaemia disorders we still have to explain why the globin chains are synthesized either at a reduced rate or not at all. Though family studies indicate that the thalassaemia mutations must be at or close to the structural genes which control the α - and β -chains this tells us nothing about their nature. A glance at fig. 2 in part I⁸ shows that there are innumerable places at which protein synthesis could be faulty in the thalassaemia syndromes. Though all these potential defects must be traced back eventually to the DNA of the affected gene it is clear that the abnormality could be expressed either at the translational stage or within the nucleus at the level of transcription of mRNA or at the gene itself.

During the last few years it has been possible to use some of the newer methods of molecular biology to study these problems. Because it is easier to study the cytoplasm of a cell than its nucleus the earliest experiments examined the processes of globin-chain initiation, translation, and termination. With the development of methods for studying mRNA and DNA the trail has gradually led back to the globin genes.

ASSEMBLY OF GLOBIN CHAINS IN THALASSAEMIA

In 1961 Dintzis⁹ showed that if rabbit reticulocytes—cells which are still actively synthesizing haemoglobin—are incubated with radioactive amino-acids for short periods and the globin chains

are then isolated and split up into small fragments it is possible by measuring the distribution of radioactivity among these fragments to estimate the time it takes to make a chain and hence to translate a globin mRNA. Using a similar approach with human reticulocytes Clegg *et al.*¹⁰ showed that it takes about 90 seconds to assemble a human β -chain at 37°C and that it takes a similar time to synthesize the β -chains of children with β -thalassaemia. These experiments ruled out a defect in the processes of mRNA translation or termination as a basis for this form of β -thalassaemia and suggested that the disease must result either from an abnormality of chain initiation or from a deficiency of mRNA for the affected chain. Later Nathan *et al.*¹¹ examined the numbers of ribosomes on the α - and β -mRNAs of thalassaemic cells, reasoning that if there was a delay in the process of chain initiation there would be fewer ribosomes than normal on each β -chain mRNA. In fact this was not the case and therefore it seems that the process of chain initiation is normal.¹¹

These experiments all suggested that a deficiency of mRNA occurs in thalassaemia but further progress awaited the development of methods for the isolation and analysis of human mRNA, which became available only about four years ago.

ISOLATION AND ASSAY OF HUMAN MESSENGER RNA

The major technical developments which have led to the isolation of mammalian mRNA have been made only in the last few years.¹²⁻¹³ Polysomes—that is, mRNA and ribosomes—can be isolated from reticulocytes by a variety of techniques. If they are disrupted the resulting mixture contains several types of RNA of different molecular sizes—ribosomal RNA, tRNA, and mRNA—and these can be separated by centrifugation through sucrose solutions of different strengths. It has been found, using a variety of animal reticulocytes, that a fraction which sediments at about 9S has the characteristics of mRNA. Neinhuis and Anderson¹⁴ and Benz and Forget¹⁵ were the first to isolate a human mRNA in this way and to show that if it is added in excess to a protein-synthesizing preparation made from rabbit reticulocytes or tumour cells it will direct the synthesis of human α - and β -chains. Using this approach they showed that a defect in β -chain production can be reproduced in a variety of heterologous systems using 9S mRNA from thalassaemic reticulocytes. These results have been confirmed¹⁶ and other assay systems have been developed for the study of human mRNA. Two of the most novel are the frog oocyte and wheatgerm, both of which translate human 9S mRNA and show thalassaemic chain imbalance when thalassaemic mRNA is injected into them.¹⁷ These experiments all provide evidence that there is either a deficiency of mRNA or an abnormal mRNA in thalassaemic red cells. To distinguish between these possibilities it became necessary to measure the amount of mRNA in human cells. This became possible with the development of techniques for synthesizing DNA on an RNA template.

SYNTHESIS OF DNA ON RNA TEMPLATE

Temin¹⁷ predicted that there must be enzymes capable of using RNA as a template for DNA synthesis. Such an enzyme was discovered independently by Baltimore¹⁸ and Temin and Mizurani²⁰ and since then has been isolated from a variety of tumour viruses and can be obtained in adequate amounts for experimental purposes from such sources as the avian myeloblastosis virus. These enzymes, known as reverse transcriptases, catalyse a reaction in which a form of DNA called complementary or cDNA is manufactured on an mRNA template viz:

Kacian *et al.*²¹ and Houseman *et al.*²² have made use of this reaction to estimate amounts of mRNA in thalassaemic red cells. Using human mRNAs as a template they produced radioactive cDNAs and used these as probes to measure the amount of

mRNA in thalassaemic cells. When DNA is heated the strands dissociate and when it is cooled they reanneal. If this is done in the presence of mRNA the latter will bind to the DNA to produce a double stranded DNA/RNA helix if there are complementary sequences present. Thus using radioactive cDNA made from human α - and β -chain mRNA it is possible to estimate the amount of mRNA in thalassaemic reticulocytes by measuring the amount of mRNA required to saturate the α - or β -cDNA. It turns out that in β -thalassaemic red-cell precursors there is a deficiency of β -mRNA and in α -thalassaemic red-cell precursors there is a deficiency of α -mRNA, when examined by the cDNA/mRNA hybridization technique.

Taken together all these experiments provide strong evidence for a deficiency of mRNA in all forms of thalassaemia looked at to date. Quite recently the molecular basis for this finding has been worked out in one common form of α -thalassaemia.

DELETION OF THE α -CHAIN GENES AS A CAUSE OF A SEVERE FORM OF α -THALASSAEMIA

The severest form of α -thalassaemia presents clinically as hydrops fetalis. Affected babies are stillborn and their haemoglobin consists entirely of Hb Bart's. This condition, called the Hb Bart's hydrops fetalis syndrome, is a frequent cause of stillbirth in south-east Asia. These babies make no α -chains²² and have no α -chain mRNA.²⁴ Do these babies have any α -chain genes? This question has been answered recently by the technique of DNA/DNA hybridization (fig. 1). Several groups of workers have shown that if total DNA is prepared from animal tissues and hybridized in large excess to cDNA made from haemoglobin mRNA it is possible to measure the number of globin-gene copies present in the total DNA.²⁵⁻²⁸ This approach has been applied to the problem of the haemoglobin Bart's hydrops fetalis syndrome by Ottolenghi *et al.*²⁷ DNA was prepared from the liver and spleen taken at necropsy from one of these babies, and the hybridization patterns with various human cDNAs prepared from globin mRNA were compared

with those of similar cDNAs with normal human DNA. The answer was clear cut. The DNA prepared from the liver of the hydropic babies seemed to contain no α -chain genes and, therefore, the disorder must result from a deletion of these genes.

This experiment was the first demonstration that a human disease can result from a gene deletion and provided the first definite molecular basis for a common form of thalassaemia—the severe α -thalassaemia gene of south-east Asia.

STRUCTURAL HAEMOGLOBINS WHICH CAUSE CLINICAL PICTURE OF THALASSAEMIA

Until recently it was thought that, except for the rare Lepore haemoglobins, there are no structural variants which cause the clinical picture of thalassaemia. This is not the case, however, and during the last few years an intriguing type of human haemoglobin variant called a chain-termination mutant has been discovered. It turns out that these are responsible for much thalassaemia in several population groups. This type of mutation was discovered during a study of families with Hb-H disease.²⁹ This is a form of α -thalassaemia which results from the inheritance of two genes: the severe α -thalassaemia gene which is now known to result from a deletion of the α -chain loci (see above) and a milder gene which results in the production of α -chains at a reduced rate. In a Jamaican family with Hb-H disease trace amounts of a slowly migrating haemoglobin component were discovered; this was subsequently named Hb Constant Spring (Constant Spring being the suburb of Kingston where the family lived). This variant was found to have elongated α -chains which are normal up to position 141 and then have 31 extra residues attached to the end, the first of which, at position 142, is glutamine. This strange haemoglobin was thought initially to be unique to one particular family but later was shown to occur commonly throughout south-east Asia: indeed, it is now estimated that it occurs in 4% of the population of Thailand and is probably equally common in the Chinese and Malay populations of Malaysia.²⁹⁻³⁰ It also has been found in Greece.

It was suggested that Hb Constant Spring has arisen in just the same way as the many other variants—that is, by a single base change in a haemoglobin gene. In this case, however, the substitution is in the terminating codon UAA, which has changed to CAA, which is the code word for glutamine.³¹ Thus the chain instead of ending at position 141 has glutamine at 142. It was then necessary to postulate that there is mRNA after the terminating codon which is not normally translated. In the case of Hb Constant Spring this extra material is translated until another "stop" codon is reached and so an elongated chain is produced. This somewhat fanciful explanation has been confirmed recently by the discovery of two further mutant haemoglobins whose structures throw considerable light on the structure of the human α -chain mRNA. The first of these is Hb Wayne.³² Like Hb Constant Spring it has an elongated α -chain but in this case the chain is normal only up to position 138 and then its structure changes and it ends at position 146 with a terminating codon at 147. This variant seems to have resulted from a process known as a "frame shift," a mutational event known to the microbial geneticist for some years but never having been seen before in human genetics. A frame shift results from the loss of a single base so that the code words get displaced; everything is still read in threes but moved along one as it were. A glance at fig. 2 will indicate that Hb Wayne can be explained by a loss of one base in the codon which normally codes for lysine at position 138. This results in all the bases being moved along one with the production of completely new sequence. If this base is "put back" the code words for the end of the α -chain and for the first part of the extra α -chain material of Hb Constant Spring can be obtained. This remarkable finding is too much to put down to coincidence and must indicate that the normal chain-terminating codon is UAA and

FIG. 1—Model showing design of experiment to answer question whether there are any α -chain genes in DNA of stillborn infants with Hb Bart's Hydrops syndrome.

FIG. 2—Structures of critical parts of α -chains of Hbs Constant Spring (C.S.), Icaria, and Wayne. Genetic code for normal terminating part of α -chain is shown, and alteration in code words which produce Hbs Icaria and Constant Spring are indicated at position 142. Hb Wayne sequence is obtained by removing a single base at position 138 and moving all code words along one, producing a new terminating codon UAG at position 147. of missing code word is replaced at position 138 sequence of end of α -chain and first part of Hb Constant Spring is obtained (see text).³³

that there is extra genetic material that is not usually translated at the end of the human α -chain mRNA. It also tells us that human beings can use another codon, UAG, for terminating a peptide chain.

When Hb Constant Spring was discovered it was predicted that similar haemoglobins might exist which are identical in structure except that they have amino-acids other than glutamine at position 142. This follows because it is theoretically possible for a single base change in the chain-terminating codon to code for several amino-acids other than glutamine. This prediction has been borne out in the last few months with the discovery of the Hb Icaria. This variant is exactly the same as Hb Constant Spring except that instead of glutamine at position 142 it has lysine.³³ The structures of Hbs Constant Spring, Wayne, and Icaria, whose discovery has told us so many fascinating things about human mRNA, are summarized in fig. 2. It seems that when the extra genetic material is translated α -chains are produced at a reduced rate, and therefore the mutations of Hbs Constant Spring and Icaria can produce the clinical picture of thalassaemia.

Thoughts for the future

The human haemoglobin story as it has unfolded has revealed an extraordinary diversity of mutational events which can produce clinical disorders. These have included point mutations, triplet deletions, additions or insertions, total-gene deletions, chain-termination mutants, frame shifts, and abnormal cross-over products. Much still remains to be done, however. In particular we still do not know why there is a deficiency of mRNA for the β -chains in the red-cell precursors of patients with the common forms of β -thalassaemia. We now seem to have the tools to tackle this problem and it will be surprising if the answers are not forthcoming in the next few years.

What then are the benefits of this new and sophisticated knowledge to patients with these common genetic disorders? A cynical answer might be that we can do little more for patients with sickle-cell anaemia and β -thalassaemia than could Herrick or Cooley when they first described these diseases in the 1920s. Indeed, such a view might not seem unreasonable when after many years of intensive study at least one of the common

thalassaemia disorders turns out to be due to a total gene deletion for which little can be done other than to hope for some form of divine intervention.

I do not subscribe to these gloomy views, however. We have learned much already from the molecular approach to the haemoglobin disorders. Thus studies of haemoglobin synthesis have provided us with a clear picture of the pathophysiology of the thalassaemia syndromes and have shown us how best to manage them symptomatically. The development of simple techniques for separating globin chains, on which much of this work is based, has lead directly to promising methods for the antenatal diagnosis of sickle-cell anaemia and possibly for thalassaemia, which should be available within the next year or so.³⁴ Furthermore, the pathophysiological studies have provided clear evidence that further reactivation of the γ -chain genes could protect both sickle and β -thalassaemic cells from the action of the primary mutation. Thus we should now concentrate our efforts in studies of the mechanism for the switch from γ - to β -chain production to see if this can be modified in any way. Since we know that reactivation of Hb F production occurs in pregnancy and in various disease states³⁵ it is not inconceivable that this switch mechanism may be amenable to manipulation. With the development of techniques for isolating human α - and β -chain genes in relatively large quantities the possibility of gene replacement must be now considered seriously, though such research should be given low priority when compared with population education, antenatal diagnosis, and fetal haemoglobin stimulation.

All the knowledge outlined in this review has been gained within a very short period of time and it would be a pessimist indeed who could say with certainty that it was going to have no further practical value within the foreseeable future.

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