GENETICS FOR THE CLINICIAN

C. A. CLARKE

M.A. M.D. F.R.C.P.

Reader in Medicine, University of Liverpool
Consultant Physician to the
United Liverpool Hospitals (The Royal Infirmary)
and to the Liverpool Regional Hospital Board
(Broadgreen Hospital)

PREFACE

I have written this book with the sole object of trying to arouse in clinicians some curiosity about genetics. This I felt necessary partly because not enough of us are aware of how relevant the subject can be to ordinary clinical work and partly because some knowledge of genetic disciplines is surprisingly useful in assessing more accurately the role of environmental factors in disease. But the trouble with genetics is to get started. The simpler rules seem only to apply to disorders which we rarely see and those more complicated are difficult to understand without an effort. We look around us, are reassured by the observation that many clinicians have risen to great eminence without any knowledge of genetics, and thus comforted we close our books. Nevertheless we should be humble. Our knowledge of why people develop common diseases (and why they frequently recover from them) is most fragmentary and much of medicine remains at the Hippocratic descriptive level, succinctly described by my Professor as the ability to recognize an elephant through having seen one before. To progress further it is essential that the problems of ordinary disease be recognized as an exercise in applied biology to be tackled on a wide front with little expectation of finding a single cause but rather in the hope of disentangling subtle combinations of factors some of which may be genetic—and in fact this pattern of multiple aetiologies is beginning to emerge.

A few, particularly those mathematically gifted, can take to genetics without difficulty but for the majority the best way of learning the subject is to breed some animal or plant because then it is soon evident why unaccustomed techniques are necessary to deal with the information which accumulates. Medical men in the past have often been good naturalists and it may in fact be rather a small step to utilize information from an outside interest for the benefit of patients and their families—and for me this happened through an interest in breeding butterflies. A chance observation, together with great good fortune in my friends, led to a rather sudden extension of the work and then the application of the principles I was learning to medical research. The channelling of the knowledge in the right direction was due directly to the E. B. Ford school of genetics at Oxford and the link between insects and Man lay in the understanding of polymorphism; once this had been mastered ready

access to big hospital populations meant that any common character could be investigated on a large scale.

This book is necessarily coloured by the way I was introduced to genetics and is made up partly of what I personally found it essential to learn and partly of those subjects in which I am particularly interested. It is in no sense comprehensive and is something of a miscellany, but with the help of the glossary each chapter can be read independently. Though everything has been published elsewhere yet the clinical applications have not, so far as I know, been brought together before.

As a professional clinician and only a Sunday geneticist I have had to turn to many for assistance. Foremost among these has been Dr P. M. Sheppard, Reader in Genetics in the University of Liverpool. He has read the entire manuscript, made numerous suggestions and generally done his best to keep me on the right lines. His co-operation with me both in the butterfly and medical work has, I think, demonstrated how fruitful it can be to wed biology to medicine. I am also extremely grateful to my colleague Dr D. A. Price Evans, Lecturer in the Department of Medicine, who has not only read most of the chapters and given me much valuable advice particularly on biochemical matters, but has also often discussed with me what information would be of interest to those whose work is primarily clinical. Dr R. B. McConnell was a founder member of the Liverpool team and we run in harness. He has given me much help, and his knowledge both of gastroenterology and serology was invaluable when writing the chapter on Heredity and the Gastrointestinal Tract. My thanks are also due to Dr J. C. Woodrow, Lecturer in the Department of Medicine, for his assistance with the genetics of connective tissue disorders; to Dr Ronald Finn for help with the erythroblastosis section; to Dr F. D. Kitchin for commenting on Inborn Errors of Metabolism; and to Dr. R. Harris, Darwin Fellow, Eugenics Society, for much support at the page proof stage.

It is a great pleasure to record how much I appreciate the way in which my Professor, Lord Cohen of Birkenhead, has sponsored the genetic research of his Department. I am very grateful for his encouragement in the writing of this book and for his helpful suggestions for the opening chapter.

Expert advice has been available on special subjects from colleagues in other Faculties and Departments and I owe much to Dr Stanley Walker, Lecturer in Genetics, for spending a great deal of time with me over the Chromosomes of Man, and to him and to Dr B. S. Cox for some useful comments on the Nature of the Gene. To Dr W. Kulke of the Isotope

Unit, the Liverpool Radium Institute, I am greatly indebted for reading and criticizing the chapter on the Biological Effects of Ionizing Radiations.

I must make special mention of the section on Elementary Statistics. This (in order to avoid undue alarm) has illogically been put at the end of the book, and it may be some comfort to O level mathematicians to know that the author is one of them and the chapter was most troublesome to compose. It was first scrutinized by Dr Sheppard and Dr Price Evans (who helped greatly by making the examples medical ones) and finally gone through by Dr M. C. K. Tweedie, Lecturer in Mathematical Statistics. He endured it with fortitude and was kindness itself in explaining to me some of my most glaring mistakes.

My thanks are due to Miss S. M. Manning and Miss M. J. Taylor who between them typed most of the manuscript and helped with the glossary and index, all with great cheerfulness, and to Miss Gwenllian Thomas for her skill and care in drawing the diagrams and pedigrees.

No matter how expert one's advisers, the book had to be written, and it is abundantly clear that it would never have materialized had it not been for the immense amount of hard work done by my wife, who read, abstracted and checked the references, unravelled for me some most puzzling problems, made rough drafts of many chapters, discussed all the subject matter, fortified morale and somehow maintained a sense of humour throughout.

Finally, I must express my appreciation to Mr Per Saugman of Blackwell Scientific Publications for much help during the publication—his advice has been invaluable and he has shown great patience with my many shortcomings.

ACKNOWLEDGMENTS

I am particularly grateful to Dr J. A. Fraser Roberts and the Oxford University Press for permission to draw freely from the section on Genetic Counselling in their book *An Introduction to Medical Genetics* (1959), for my chapter 'The Heredity Clinic'.

I wish to thank the McGraw Hill Book Company and the Editors of *The Metabolic Basis of Inherited Disease* (1960) for allowing me to use the arrangement of their book for my chapter on 'Inborn Errors of Metabolism'.

I am much indebted to the British Council and the Editor of the *British Medical Bulletin* for permission to use, almost in its entirety, the article by Dr D. A. Price Evans and myself on Pharmacogenetics (*Bulletin*, September, 1961), for the chapter on the same subject in this book; to the Editor of the *British Medical Journal* for a similar courtesy in respect of the paper by my colleagues and myself (*Brit. med. J.*, 1961, I, 1486) for the chapter 'Protective Mechanisms in Rh Haemolytic Disease'; and to the Pergamon Press for allowing me to use material from my chapter on 'Biological Fitness' in *Natural Selection in Human Populations* for Chapter VI in this book.

Dr D. G. Harnden and Messrs Churchill kindly granted me permission to use their very concise section on the identification of chromosomes from *Recent Advances in Human Genetics* (1961) edited by Professor L. S. Penrose, F.R.S., for my Chapter II, 'The Chromosomes of Man'.

I am also very grateful to Professor V. A. McKusick and the C. V. Mosby Press for allowing me to utilize material from *Heritable Disorders of Connective Tissue*, 2nd edition (1960), and for the loan of photographs.

I wish to thank the following authors and publishers for permission to reproduce material in the various chapters listed below.

Chapter I

Dr E. B. Ford, F.R.S., and Messrs Methuen for the quotation from *Genetics for Medical Students* (1956).

Dr J. D. Watson and Dr F. H. C. Crick and the Editor of the Cold Spring Harbour Symposium for Figure 2.

Chapter II

Dr Winston Evans, David Lewis Northern Hospital, Liverpool, for Plate I.

Professor C. P. Swanson and Messrs Prentice Hall for Figure 2.

Dr S. Walker, Sub-Department of Genetics, Liverpool University, for Plates II and III.

Dr C. O. Carter and his co-authors, and the Editor of the *Lancet* for Figure 4.

Chapter III

Dr J. A. Fraser Roberts and the Oxford University Press for Table I.

Chapter IV

Mr Wilfrid Lee for the photographs of Swallowtail butterflies.

Dr R. R. Race, F.R.S., Dr Ruth Sanger and Blackwell Scientific Publications for Figure 1.

Chapter VII

Mr C. D. B. Ellis and Messrs Blackwell for the poem quoted at the beginning of the chapter.

Chapter VIII

Mr Eric Norman, Department of Radio-diagnosis, University of Liverpool, for Plates I, II and III.

Dr J. H. Renwick, Dr Sylvia Lawler and the Editor of the *Annals of Human Genetics* for Pedigree II; the Editor of the *Annals of Human Genetics* for Pedigrees III, IV and V.

Professor Curt Stern and Messrs Freeman and Company, San Francisco, for Pedigrees VI, VII and VIII (from *Principles of Human Genetics*. 2nd edition, 1960).

The Editor of the *Quarterly Journal of Medicine* for Pedigree IX.

Dr J. A. Fraser Roberts and the Editor of the *British Medical Bulletin* for Table I.

Chapter IX

Dr R. B. McConnell and the Editor of the *British Medical Journal* for the section on Crohn's disease.

Chapter X

Sir Robert Platt and the Editor of the *Lancet* for Figures 1 and 2.

Dr W. E. Miall, Dr P. D. Oldham and the Editor of *Clinical Science* for Table I and for Figures 3 and 4.

Sir George Pickering and his co-authors and the Editor of the *Lancet* for Figure 5.

Chapter XI

Professor V. A. McKusick and the C.V. Mosby Company for Plates III, IV, V and X, the Editor of the *Annals of Internal Medicine* for Plate II and the Editor of the *Bulletin of the New York Academy of Medicine* for Plate VI, both also lent by Professor V. A. McKusick.

Dr G. M. Berlyne and the Medical Illustration Department of the Manchester Royal Infirmary for Plates VII, VIII and IX.

Dr M. A. Orgryzlo and the Editor of the *Canadian Medical Association Journal* for Plates XI and XII.

Sir Macfarlane Burnet, F.R.S., and the Editor of the *British Medical Journal* for Figure 1.

Chapter XIII

Dr H. Lehmann, Dr J. A. M. Ager and the McGraw Hill Book Company for the world distribution map of the haemoglobins, and Dr T. B. Fitzpatrick and the same publishers for the table on albinism. Both map and table are from *The Metabolic Basis of Inherited Disease* (1960), ed. by Drs Stanbury, Wyngaarden and Fredrickson.

Chapter XVI

Dr J. H. Renwick and his co-authors, and the Editor of the *American Journal of Human Genetics* for the phenylalanine tolerance test histogram.

Dr H. F. Falls and Dr C. W. Cotterman and the Editor of the *Archives of Ophthalmology* for Plates Ia and Ib.

Dr H. Foy and *Transactions of the Royal Society of Tropical Medicine and Hygiene* for Plate II.

Springer-Verlag, Berlin, for the mental deficiency table by C. Brugger (1939), *Hdb. Erbbiolog. d. Menschen*, ed. by Just, Bauer and Hanhart, vol. 2, page 294.

Chapter XVII

Dr George Gibson and the Editor of the *British Medical Journal* for Figure 5.

The Editor of the *British Medical Journal* for Figure 7.

Dr D. J. Finney and Messrs Einar Munksgaard, Copenhagen, for Figures 9 and 10.

Dr M. J. Moroney and Penguin Books, Ltd., for Figure 12 (from *Facts from Figures*).

Dr. S. C. Truelove, Oxford University Press, for part of the material from the section on Clinical Trials.

Dr P. Armitage and the Editor of the *Quarterly Journal of Medicine* for Figure 15.

Professor Sir Ronald Fisher, F.R.S., Dr Frank Yates and Messrs Oliver and Boyd, Ltd, Edinburgh, for the tables from their book *Statistical Tables for Biological, Agricultural and Medical Research*.

CONTENTS

CHAPTER ONE

THE NATURE OF THE GENE

To think of Gregor Mendel is for many to recall only some dull and simple ratios, the result of breeding garden peas. There is often failure to realize that the fundamental importance of his work was the discovery that the characters of an organism are inherited as discrete units and that each retains its particulate nature from generation to generation. This still remains a good approximation of what geneticists believe today, yet a hundred years ago Darwin was ignorant of it and thought that inheritance was blended. He appreciated that blending would lead to uniformity whereas all around he saw variability but he explained the difficulty by assuming that mutations occurred very frequently—a view which we now know to be untrue. Mendel's work showed conclusively that blending of the units of inheritance—the genes—did *not* occur, for though the first filial (F1) generation often presented an intermediate appearance yet in the second filial (F2) generation the genes would segregate and the parental characters reappear unchanged. It is *genes* which do not blend—characters may be and often are intermediate.

When Mendel's work was rediscovered in 1900 much more was known about the chromosomes and it was soon obvious that they provided exactly the right mechanism necessary for carrying the genes and for ensuring their segregation. Ford (1956) has pointed out a close parallel between the behaviour of the chromosomes as seen under the microscope and that of the genes whose action can be inferred by tracing the inheritance of the characters which they control. We cannot do better than quote a striking passage from his book: 'The genes are present in pairs (allelomorphs) as are the chromosomes (homologous chromosomes). The members of these pairs, both of genes and of chromosomes, are derived respectively from the two parents. Consequent upon Mendelian segregation, the genes constituting the allelomorphs separate from one another and pass into different gametes, as do the members of the homologous pairs of chromosomes, owing to meiosis. The gametes then contain one member only of the pairs both of genes and of chromosomes; but these are restored by the additive nature of fertilization'. (Ford, 1956.)

There is overwhelming evidence that genes are situated on the chromosomes. For example, if we look at the salivary gland of *Drosophila* we see

on each giant chromosome transverse bands, some wide and some narrow, the patterns being specific for any given chromosome. These are the chromomeres and they are certainly associated with the genes since irradiation of *Drosophila* frequently results in a mutation known as 'notched wing' which is often (though not always) paralleled by absence of a specific chromomere in a specific rod-shaped chromosome (Stern, 1960). It is necessary now to consider whether the arrangement of the genes is of importance. That this is the case is shown again in *Drosophila* where observable inversions or translocations (p. 21) can take place, with the result that some of the genes must have altered their position with respect to some of their neighbours. When this happens their manifestations may also be altered, a phenomenon known as the 'position effect'.

We next have to see whether the action of the genes can be demonstrated to have a physico-chemical basis, and there is much evidence to show that this is so. Let us take as our example eye-colour in the European flour moth. Normally the insect has black eyes but occasionally a recessive mutant occurs in which the pigment is red. If wild-type organs such as testis, ovary or brain are implanted into 'red' larvae the eyes of the resulting moth are no longer red but black. Similar results are obtained if 'red' organs are implanted into wild-type larvae, the eyes of the mature insects again being black. Furthermore, on crossing 'implanted' mutant females to mutant males the eye-colour is again wild-type, although normally the mating will produce all red-eyed insects (Kühn *et al.*, 1935). The reason for these results is that the wild-type allele gives rise to a diffusible hormone (kynurenine) which enables tryptophane to be oxidized to the dark pigment whereas in the mutant the oxidative step from tryptophane to kynurenine is blocked, so that red pigment is formed instead of dark.

Such chemical evidence can readily be paralleled in Man, where there are many genetically controlled enzyme defects responsible for metabolic 'blocks' which often show themselves as specific diseases (see Chapter XIII). Nevertheless, we have to remember that both in the flour moth and in Man biochemical reactions are specific to particular cells but they must have received appropriate instructions and the evidence is that these originate in the nuclei. How this takes place must next be considered, and we must turn to microbiology for our evidence.

It will be remembered that certain strains of *pneumococci* form 'smooth' (S) colonies and that these tend to be virulent and to have capsules with differing antigenic properties. Conversely, the rough (R) strains are non-

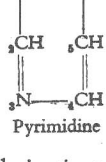
encapsulated and less pathogenic. As long ago as 1928 Griffith showed that when mice were injected with living (R) *pneumococci* mixed with killed (S) strains, living (S) *pneumococci* could be recovered from the animals. In other words, some of the (R) *pneumococci* had been 'transformed' to the (S) type and the latter continued to reproduce themselves in subsequent generations 'as if a new gene had been added to the genetic make-up of the receptor cells' (Zamenhof, 1959). Later it was shown that the same transformation could be demonstrated *in vitro* and then even that cell-free extracts of the killed (S) cells could also bring about the same phenomenon. The final link was provided by Avery and his colleagues (1944) who showed that the transforming principle was in fact deoxyribonucleic acid (DNA).

Viruses next come into the picture. Certain of these (the bacteriophages) are capable of so increasing the permeability of some bacterial cells that the latter disintegrate (lysis). Working on *Salmonella*, Lederberg and his colleagues (1951) found that the lysate contained a filterable agent which was capable of inducing the heritable properties of the lysed *Salmonella* in other genetically different strains of the same bacterium. However, only a small proportion of the recipient cells acquire the new characters. This phenomenon is known as *transduction*. What exactly happens is not clear but it seems that new DNA is introduced into the recipient cells and becomes incorporated in their genetic make-up. The likelihood is that fragments of bacterial chromosome are transported from one cell to another by the vector.

We must now consider the molecular structure of DNA, since although chromosomes, chromomeres, inversions and translocations all give information about the nature of genes at the microscopic level, yet in the transforming principle in bacteria we are dealing with a solution of DNA where all sequences of individual genes are entirely destroyed. Clearly we must consider this remarkable substance in more detail since it now seems indisputable that it is the most important part of the heritable material of all living things, i.e., genes and various arrangements of DNA are synonymous.

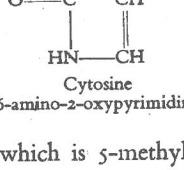
Let us begin with some M.B. biochemistry. The nuclei of cells contain nucleic acids existing in association with protein as nucleo-protein. If we wish to know the composition of nucleic acids we carry out the usual chemical process of hydrolysis with a weak acid and we find that they are made up of three principal constituents—(1) phosphoric acid, (2) a sugar (pentose) and (3) organic nitrogenous bases known as pyrimidines or purines. Both of these last will be remembered in connexion with gout,

since the end-product of their metabolism is uric acid and they are present in food, particularly in meat, sweetbread and other cellular organs. Pyrimidine itself has the following structure and the conventional numberings are added:



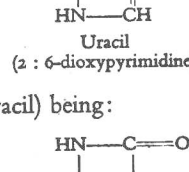
Pyrimidine

The first of the two important derivatives of pyrimidine is cytosine, which is 6-amino-2-oxypyrimidine, thus:



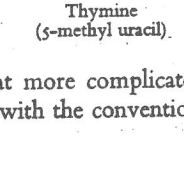
Cytosine
(6-amino-2-oxypyrimidine)

The second is thymine, which is 5-methyl uracil, uracil being 2 : 6-dioxypyrimidine, thus:



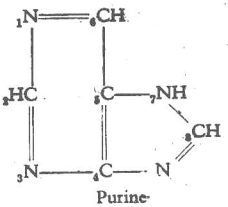
Uracil
(2 : 6-dioxypyrimidine)

and thymine (5-methyl uracil) being:



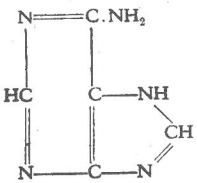
Thymine
(5-methyl uracil)

The purines are somewhat more complicated, purine itself having the following formula (again with the conventional numbers added):

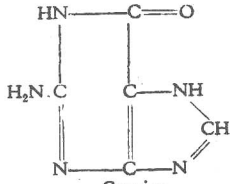


Purine

The only purines found in nucleic acid are adenine, which is 6-amino-purine, and guanine, which is 2-amino-6-oxypurine; the formulae for these are given below:



Adenine
(6-aminopurine)



Guanine
(2-amino-6-oxypurine)

Having established the basic chemical formula of DNA we must next consider the arrangements in the molecule and the elegant Watson-Crick model (1953) is now generally accepted as the correct explanation. The first building block is a nucleotide, which is composed of a molecule of one of the nitrogenous bases, one of the pentose sugar and one of phosphoric acid. These polymerize to form a molecule of DNA and Fig. I, 1 shows the way in which four nucleotides can be arranged,

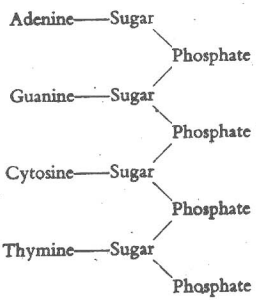


Figure I, 1. The arrangement of four nucleotides in the DNA molecule.

The polymer is one of the two chains of which a molecule of DNA is composed. The other chain has similar components but it is very important to remember that a purine base always pairs with a pyrimidine. The reason for this is that purines have too big and pyrimidines too small a molecule to pair with each other, the double chain always being constant in width. Each purine always pairs with the same pyrimidine, adenine with thymine and cytosine with guanine. The double chain consists of one giant molecule of DNA—it may have as many as 10,000 purine: pyrimidine pairs and its molecular weight is about 6,000,000. Furthermore, it has been estimated that its length is about 30,000 Ångstroms (one Ångstrom unit is a hundred millionth of a centimetre), and its thickness is 20 Ångstroms—that is, it is more than 1,000 times as long as it is thick.

The pairs of bases in the molecule of DNA can follow one another in any order and the ratio of adenine or thymine to guanine or cytosine is different in different species. The ratio of total purine to total pyrimidine is, however, always unity.

In the Watson-Crick model the nucleotides, when paired, have a backbone of the pentose-phosphate chain, and the nitrogenous bases are directed inwards and joined together by hydrogen bonds. This is shown below in Fig. I, 2.

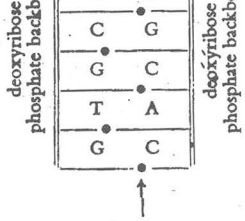


Figure I, 2. Hydrogen bonds.

The final feature of the model is that the two chains are twisted round one another to form the well-known double helix, resembling a spiral staircase. This is shown in Fig. I, 3.

The evidence for the helix comes from X-ray diffraction, which tells us about the arrangement in space of the atoms inside the molecules of crystals, and is, therefore, applicable to DNA when it is in this form.

Diffraction studies showed that the repeats in the pattern came at much longer intervals than the repeats when DNA was examined chemically. This led Watson and Crick to suggest that the X-rays were only revealing

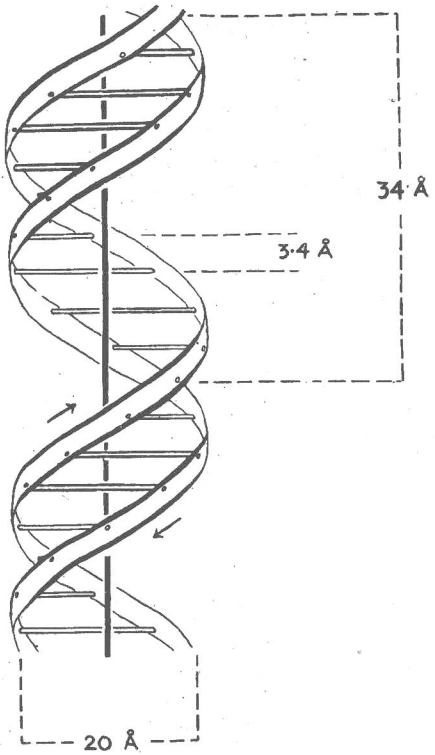


Figure I, 3. Diagram representing the double helix of DNA, giving dimensions in Ångstrom units. The two ribbons symbolise the two phosphate-sugar chains and the horizontal rods the paths of the bases holding the chains together. The arrows show that the sequence of bases goes one way in one chain and the opposite way in the other. The vertical line represents the axis of the molecule.

that proportion of links which was seen from the same angle, a result which would be expected if the chain were coiled in a helix (Crick, 1955).

Any substance which forms the heritable material must be stable,