Medical Genetics:

Principles and Practice

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SECOND EDITION

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Preface

The second edition of *Medical Genetics* represents a major revision dictated by the rapid accumulation of knowledge in basic and clinical genetics. Indeed, in retrospect, the logistics of the extensive rewriting would perhaps have been more easily accomplished by submitting an entirely new manuscript. However, many of the illustrations and a considerable amount of text did not become obsolescent in the relatively short interval between editions. The references are, as in the first edition, not intended to be comprehensive, but rather to offer supplementary information. Sometimes an original observation is cited; sometimes a more recent review is selected. Clearly, the credit due many investigators for important contributions has been sacrificed for the sake of maintaining a textbook of manageable size.

For more detailed information on genetic theory, diagnosis, and treatment the reader is referred to more extensive texts of genetics, pediatrics, or medicine to be kept in the library or office. We hope that this book will continue to find its place in medical school classrooms, clinics, and counseling centers. We also hope that the appearance of this second edition in proximity to the offering of the first examination of the American Board of Medical Genetics will provide up-to-date information that will be useful for review.

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Heredity and Disease

Chapter 1

Heritability of Diseases and Traits

BUT THIS DISEASE SEEMS TO ME TO BE NO MORE DIVINE THAN OTHERS. . . ITS ORIGIN IS HEREDITARY LIKE THAT OF OTHER DISEASES. . . WHAT IS TO HINDER IT FROM HAPPENING THAT WHERE THE FATHER AND MOTHER WERE SUBJECT TO THIS DISEASE, CERTAIN OF THEIR OFFSPRING SHOULD BE AFFECTED ALSO?

HIPPOCRATES: ON THE SACRED DISEASE.

From the beginning of the history of Western medicine, the heritability of physical traits and diseases has been recognized. Hippocrates not only observed that blue eyes and baldness ran in families, but that diseases such as epilepsy followed a similar pattern. Before the early twentieth century, inheritance was considered to be a blending, a continuous variation, and this is probably what Hippocrates had in mind. However, the emphasis shifted away from blended inheritance following the rediscovery of Mendel[3] and unit inheritance and the locating of the hereditary particles, the genes, in chromosomes. Indeed, among the earliest published examples of mendelian inheritance was the disease alkaptonuria, described by Sir Archibald Garrod in 1902.[1] A large number of diseases attributed to single mutant genes followed this remarkable observation until the current catalog of disorders considered to have a firm mendelian basis lists 1364 conditions.[2] The terms "dominant" and "recessive" entered the medical vocabulary, and many diseases which have later been demonstrated to have no true basis in mendelian inheritance still carry such labels. If a disease was presumed to have a genetic basis, an effort at mendelian interpretation was made.

A further shift in emphasis began in 1959, when the first disorders were described that could be traced to abnormalities of chromosome number. During the next few years, several more syndromes associated with a chromosomal aberration were discovered. Then, in the minds of many students (and referring physicians), the erroneous idea took root that if a disease has a genetic basis, a chromosome karyotype must be ordered to establish the diagnosis. However, the

consultant in genetics appreciates that a large percentage of the patients he is asked to see have disorders that can be attributed to neither a single mutant gene nor a chromosomal anomaly. If there is a genetic basis for these diseases, then we must return through the full circle to Hippocrates and discuss the hereditary aspect of disease in its earliest sense, that is, predisposition or diathesis.

A useful classification of diseases having a genetic background would thus be:

1. Single mutant gene (mendelian) syndromes
2. Chromosomal aberration syndromes
3. Diseases determined by multifactorial inheritance—genetic predisposition with environmental interaction.
4. Developmental abnormalities in which the environmental contribution is the major element, e.g., rubella and thalidomide syndromes.

One may ask, how does an investigator determine whether or not genetic factors are important in a disease whose etiology is unknown? Several tools are available, and these are discussed in their appropriate chapters.

First, if a disease has a genetic basis, it will occur in **familial aggregates**. This does not mean that all diseases found in more than one member of a family are necessarily genetic: take, for example, an epidemic of chickenpox or a bout of food poisoning. How then can one distinguish between familial environmental causes and genetic causes? One may begin by testing the data to see whether they fit the expectation for mendelian or multifactorial inheritance. If they do, a nongenetic cause is unlikely, although one must carefully rule out potential environmental causes.

Second, **twin studies** measuring the differences in concordance between monozygotic and dizygotic twins with respect to a given disease offer a means to determine whether the familial distribution results from genetic factors and to assess the contribution of these genetic factors to the disorder.

Finally, **animal homologies** are used to aid in the understanding of etiologic mechanisms in the human subject.

RECURRING THEMES

It is useful to state at the outset certain themes, concepts, and definitions that are so central that they must be repeated frequently in any treatment of genetics. It is likely that a reader with modest sophistication will find this discussion too elementary. We apologize, but submit that the most elementary material is not familiar to everyone and must be stated somewhere in an introductory text.

Mendel, Genes, and Chromosomes. Although this a textbook of human genetics, and our examples will be generally confined to the human subject, it is entirely appropriate that mendelian inheritance be given its first exposure in the light of original materials and methods. Laws of heredity became apparent when certain highly distinctive traits in garden peas were selected and studied by the Austrian monk, Gregor Mendel.[3] These traits are defined by what we now know to be segments of deoxyribonucleic acid (DNA), called **genes.** Genes are linked together within a larger structure, the **chromosome.** In higher organisms chromosomes come in pairs. Alternative forms of a gene exist, which are called **alleles.** Alleles occupy the same **locus** or position on homologous chromosomes. Each pair of homologous chromosomes is identical with respect to its loci (unless there is a structural anomaly). When Mendel was looking at round peas and wrinkled peas, he was looking at the alleles at the same locus on one pair of homologous chromosomes of the seven pairs in garden peas—the locus which defines a surface characteristic of the pea. In discussing one set of different chromo-

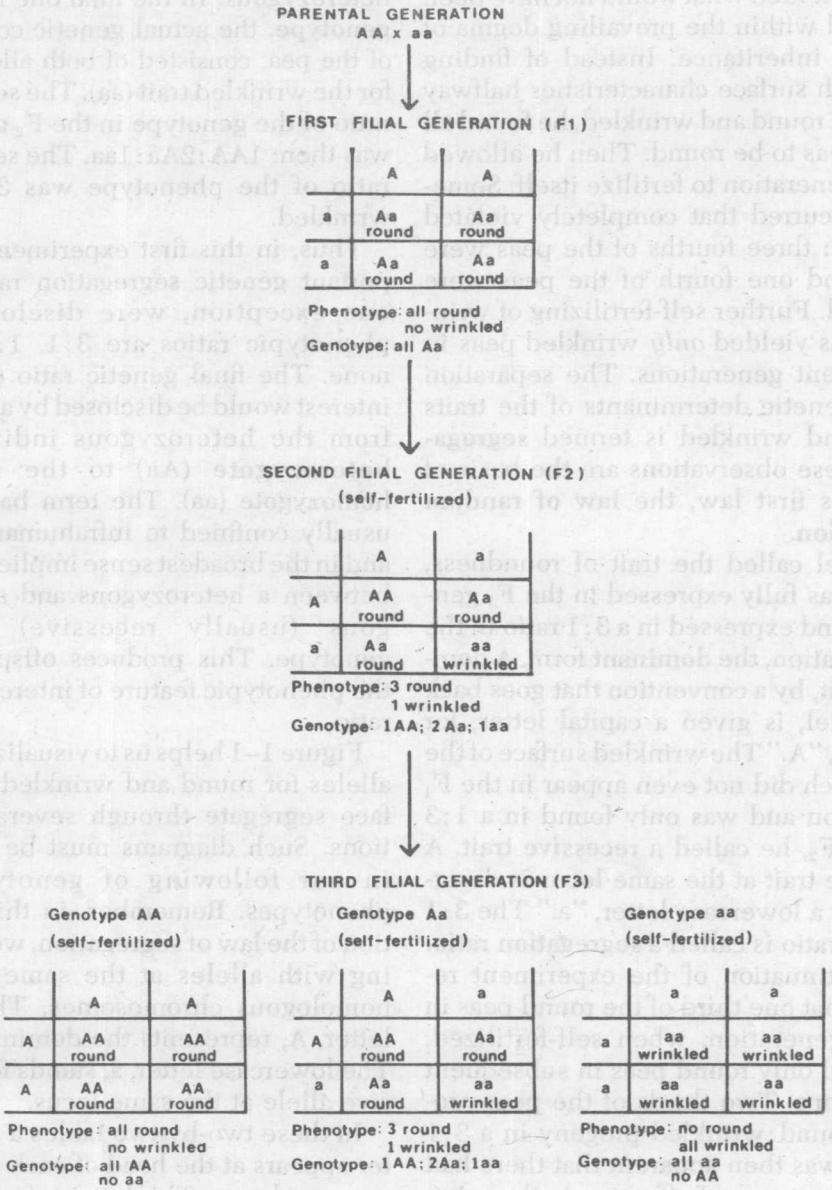

Fig. 1–1. Diagrammatic representation of the law of random segregation.

somes (7 in the garden pea and 23 in the human), the term **haploid** number is used. Actually there are 14 chromosomes in the pea and 46 in the human. These are the **diploid** numbers of chromosomes—the chromosomes that one can count and photograph under a microscope. The chromosomes may then be displayed in homologous pairs in what is called a **karyotype.**

Referring to Figure 1–1 may help the reader through the next several paragraphs. When Mendel crossed two true-breeding strains, round peas × wrinkled peas, the progeny, the F_1 (first filial) gen-

eration yielded what would not have been expected within the prevailing dogma of blended inheritance. Instead of finding peas with surface characteristics halfway between round and wrinkled, he found all of the peas to be round. Then he allowed the F_1 generation to fertilize itself. Something occurred that completely violated intuition: three fourths of the peas were round and one fourth of the peas were wrinkled. Further self-fertilizing of wrinkled peas yielded *only* wrinkled peas in subsequent generations. The separation of the genetic determinants of the traits round and wrinkled is termed **segregation.** These observations are the basis of Mendel's first law, the **law of random segregation.**

Mendel called the trait of roundness, which was fully expressed in the F_1 generation and expressed in a 3:1 ratio in the F_2 generation, the **dominant** form. A dominant trait, by a convention that goes back to Mendel, is given a capital letter, for example, "A." The wrinkled surface of the pea, which did not even appear in the F_1 generation and was only found in a 1:3 ratio in F_2, he called a **recessive** trait. A recessive trait at the same locus is designated by a lowercase letter, "a." The 3:1 (or 1:3) ratio is called a **segregation ratio.**

A continuation of the experiment revealed that one third of the round peas in the F_2 generation, when self-fertilized, produced only round peas in subsequent generations. Two thirds of the peas produced round:wrinkled progeny in a 3:1 ratio. It was then apparent that there had been three types of offspring in the original F_2 generation. In one fourth of the peas both alleles carried the genetic trait, round (AA). **Homozygous** is the term used when both alleles are identical. In one half of the progeny, a dominant "round" allele was paired with a recessive "wrinkled" allele (Aa), but the **phenotype** of the seed surface, the observable property of the pea, was round. When the two alleles are not identical, they are said to be **heterozygous.** In the final one fourth, the **genotype,** the actual genetic constitution of the pea, consisted of both alleles being for the wrinkled trait (aa). The segregation ratio of the genotype in the F_2 generation was then: 1AA:2Aa:1aa. The segregation ratio of the phenotype was 3 round:1 wrinkled.

Thus, in this first experiment, the important genetic segregation ratios, with one exception, were disclosed. The phenotypic ratios are 3:1, 1:3, all, or none. The final genetic ratio of general interest would be disclosed by a backcross from the heterozygous individual or **heterozygote** (Aa) to the recessive **homozygote** (aa). The term **backcross** is usually confined to infrahuman subjects and in the broadest sense implies a mating between a heterozygous and a homozygous (usually recessive) parental genotype. This produces offspring with the phenotypic feature of interest in a 1:1 ratio.

Figure 1–1 helps us to visualize how the alleles for round and wrinkled seed surface segregate through several generations. Such diagrams must be relied on in our following of genotypes and phenotypes. Remember, in this illustration of the law of segregation, we are dealing with alleles at the same locus on homologous chromosomes. The capital letter, A, represents the dominant allele. The lowercase letter, a, stands for a recessive allele at the same locus.

In these two-by-two tables a single letter appears at the head of each of the columns and rows. This letter is for the single allele which is transmitted in the **gamete,** the mature reproductive cell of either parent. When the male reproductive cell fertilizes the female reproductive cell, a **zygote** is formed. In the normal human somatic cell there are 46 chromosomes, and in the garden pea, 14. After fertilization, an organism grows by cell division, and the genetic information of each cell is passed to two new cells by a process

called **mitosis.** In Chapter 2 a more detailed discussion of this subject is offered. To avoid providing duplicate illustrations, the reader is asked to scan Figure 2–9 on page 20. In mitosis in the human each of the 46 chromosomes (23 pairs) divides to form new cells, and each new cell also has 46 chromosomes. But **meiosis**, which is the process by which reproductive or germ cells divide to form gametes, is different from mitosis.

In human meiosis (see Figs. 2–10, 2–11 2–12) the homologous chromosomes join together and, having joined, they may exchange genetic material before each of 23 paired chromosomes separates from its homologue and is incorporated into a daughter cell having not 46 chromosomes, but 23. The 23 chromosomes are not random but one from each of the 23 pairs. A second meiotic division then takes place which is similar to mitosis in that each of the 23 individual chromosomes divides, and this process ends with the formation of a mature gamete with 23 chromosomes. The gametes then unite at fertilization, and the original 46 chromosomes are reconstituted in the human zygote.

Back to the garden pea. If Mendel had selected traits that were all on the same chromosome, the second law, the **law of independent assortment**, would not have been readily apparent (because the traits would have tended to segregate together as **linked** alleles on the same chromosome). He was fortunate in his selection of subsequent characteristics. The gene for color of seed, yellow or green, is located on another chromosome. This permitted Mendel to recognize that peas could be yellow and wrinkled, green and round, green and wrinkled, or yellow and round. The genes for yellow and green are allelic on one pair of chromosomes and the genes for round and wrinkled are alleles at the same locus on a different chromosome.

In his original paper published in 1865, Mendel looked at seven differentiating characters in garden peas and described experiments with hybrids of other species of plants. Unfortunately, Mendel's work was about 35 years ahead of the *Zeitgeist*. The enormous importance of the studies was simply not recognized until 1900, when not one, but three investigators independently confirmed Mendel's experiments. And what happened to Mendel after the landmark discoveries that laid the foundation of the science of genetics? He did what many good researchers do. He left investigative work for administration.

BACK TO PEOPLE

As mentioned earlier, almost as soon as Mendel was rediscovered, applications of his laws of inheritance to the human subject were found. Mendel's laws remain one of the most valued stocks in trade for the geneticist dealing with humans. But, of course, the clinical geneticist is asked to see patients for several different reasons. (See Tables 1–1 and 1–2 for our recent experience in Denver.)

Often an infant or child is born with a common malformation, and the parents are concerned about the risk of recurrence. Is the malformation inherited? Is there something that the parents did to cause this problem? What is the chance that this may recur and what can be done to prevent it?

Table 1–1. Etiologic Categories of 1078 Patients Presenting for Genetic Consultation (Denver Experience 1/1/78 to 6/30/79)

Category	Number	%
Chromosomal	218	20.2
Multifactorial inheritance	171	15.9
Autosomal dominant	148	13.7
X-linked inheritance	95	8.8
Autosomal recessive	94	8.7
Environmental exposures	76	7.1
Undetermined	276	25.6

Table 1–2. Twenty-five Most Common Categories of Patients Appearing for Genetic Consultation (Denver Experience 1/1/78 to 6/30/79)

1.	Preamniocentesis counseling for maternal age	477
2.	Down syndrome	125
3.	Mental retardation and developmental delay	85
4.	Neural tube defects	79
5.	Exposure to teratogens or mutagens	68
6.	Hemophilia	58
7.	Multiple spontaneous abortions	48
8.	Cleft lip, cleft palate	44
9.	Congenital heart disease	34
10.	Multiple congenital anomalies	33
11.	Cystic fibrosis	18
12.	Marfan syndrome	15
13.	Diabetes	14
14.	Neurofibromatosis	14
15.	Hydrocephalus	12
16.	Turner syndrome	11
17.	Muscular dystrophy	11
18.	Huntington chorea	11
19.	Trisomy 13	11
20.	Ehlers-Danlos syndrome	10
21.	Consanguineous matings	10
22.	Osteogenesis imperfecta	10
23.	Trisomy 18	8
24.	Myotonic dystrophy	7
25.	Charcot-Marie-Tooth disease	7

Another category of patients referred to the clinical genetics consultant is a patient with a pattern of anomalies in search of a diagnostic label. The hope here is that naming a disease will explain it. In some cases this is true. Determining that a patient has the Marfan syndrome provides a reasonable basis for medical management, prognosis, and counseling. Often, however, suggesting a label for a group of anomalies implies a greater understanding of the disease than actually exists. The cause of the condition is uppermost in the minds of the anxious parents. Invoking a difficult-to-pronounce eponym makes the geneticist appear to be a scholar, but he is deceiving both himself and his patients unless he acknowledges the limits of his diagnostic label. Does naming this disease answer the question of etiology? Does it provide a reasonably firm basis for discussing prognosis in the patient and risk of recurrence in the family? And how precise is the diagnosis of the Balderdash syndrome, anyway? Could this be another condition entirely?

If the patient has a common malformation, the familial aspects of which have been well investigated (e.g., atrial septal defect), then meaningful genetic counseling may be offered. If the patient clearly has a specific syndrome about which there is usable etiologic and prognostic information (e.g., Hurler syndrome or 21 trisomy), it is possible for the geneticist to answer many urgent questions.

As our data base increases, so does its complexity. Heterogeneity and polymorphisms are becoming more widely recognized. In **genetic heterogeneity** the same or similar physical characteristics may be produced by different genes (loci) or different mechanisms. **Genetic polymorphism** means that there is more than one allele for a given locus (with a frequency greater than 1%). The ABO blood type is a typical example of a genetic polymorphism. It has been estimated that, in man, one third of the loci are polymorphic. Related to the concept of genetic heterogeneity is the concept of **pleiotropy,** in which a single gene or gene pair may produce multiple different effects (e.g., anomalies of the heart, the eye, and the skeleton in Marfan syndrome).

A few years ago it was possible to talk about Ehlers-Danlos syndrome as if it represented one disease with one mode of inheritance. Now we must distinguish at least four autosomal dominant, two autosomal recessive, and one X-linked form of the disease. The more we know about a disease, the more we appreciate that the disease in question may be several diseases with several separate etiologies.

Knowledge in fundamental genetics has expanded explosively during the past decade to the point that it may be considered the central and unifying biologic science. The aim of this monograph is to explore medical genetics following the map provided by investigation into the fundamental areas of genetics.

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Chapter 2

Chromosomal Basis of Heredity

THE GENERAL CONCEPTIONS HERE ADVANCED WERE EVOLVED PURELY FROM CYTOLOGICAL DATA, BEFORE THE AUTHOR HAD KNOWLEDGE OF THE MENDELIAN PRINCIPLES. . . AS WILL APPEAR HEREAFTER THEY COMPLETELY SATISFY THE CONDITIONS IN TYPICAL MENDELIAN CASES, AND IT SEEMS THAT MANY OF THE KNOWN DEVIATIONS FROM THE MENDELIAN TYPE MAY BE EXPLAINED BY EASILY CONCEIVABLE VARIATIONS FROM THE NORMAL CHROMOSOMIC PROCESSES.
WALTER S. SUTTON: THE CHROMOSOMES IN HEREDITY. BIOLOGICAL BULLETIN, 4:231, 1903.

The word *chromosome* was introduced in 1888 by Waldeyer. As is the case with many important discoveries, the early recognition of the role of the chromosome as the carrier of the information of heredity must be credited to several investigators. Working in the late nineteenth and early twentieth centuries, Roux, Boveri, Wilson and Sutton pursued a course running parallel to that followed by genetic researchers and appreciated before the rediscovery of Mendel that the chromosomes could be the ultimate dividing units and carriers of heredity. However, it was the rediscovery of Mendel that provided the catalyst for the reaction that synthesized the discoveries of cytology and genetics into the discipline of cytogenetics. It became apparent to the cytologists that the behavior of the hereditary characters of Mendel was reflected by the behavior of the chromosomes in meiosis. Sutton and Boveri independently proposed the chromosomal hypothesis of inheritance (the "Sutton-Boveri hypothesis").

The remarkable contributions to the chromosomal basis of heredity that were made over the next decades were, of necessity, derived from studies in lower animals, the drosophila proving to be a most useful subject. As early as 1910, T. H. Morgan was able to locate a specific gene locus on a specific chromosome of *Drosophila melanogaster*. The human, however, is in many ways an unsatisfactory subject for genetics research. This has been especially true in the area of

cytogenetics. It was not until 1956 that the diploid number of human chromosomes was demonstrated to be 46 by Tjio and Levan.[7] For the 33 years before this date, students of medicine and biology were taught that the human diploid complement was 48. The reason for this discrepancy was not carelessness on the part of cytogeneticists. Rather, the determination of the correct diploid number had to await the development of techniques capable of accurately revealing the human chromosomes.

Several technical advances have made the study of human chromosomes a useful clinical as well as investigative procedure. Exposing dividing fibroblasts to colchicine arrests cell division in metaphase, and employing a hypotonic solution causes the metaphase chromosomes to become more distinct. The latter technique was discovered along the frequently traveled scientific path of serendipity (when a technician mistakenly made a medium of the wrong concentration). Phytohemagglutinin, which agglutinates red blood cells, was also found to stimulate lymphocytes to divide. This permitted the culturing of peripheral blood rather than fibroblasts, resulting in greater versatility and patient acceptability. The preceding techniques contributed to the first period of rapid growth in the cytogenetics of man. In 1970 banding methods, which will be discussed later, initiated the second log-phase of growth in the study of human chromosomes.

Recognizing that the hereditary material was carried by the chromosomes did not, of course, define the nature of the unit of inheritance, which Johannsen labeled the gene. The development of this line of investigation is undertaken in Chapter 5. The chromosome itself consists of the hereditary material, deoxyribonucleic acid (DNA), organized into a complex structure along with histone and nonhistone proteins. The ultimate units of inheritance, the genes, are segments of DNA. It has been estimated by several methods that the human genome contains on the order of 50,000 structural genes—genes that determine the amino acid sequence of polypeptide chains of proteins. The amount of DNA in man is enough to make two to five million genes of average length. However, much of the DNA is **repetitive**, that is, the same sequence occurs repeatedly (from 10^2 to 10^6 times) interspersed between the unique or nonrepetitive sequences that determine structural genes. Repetitive DNA may also occur in clusters in a single area. Functions of repetitive DNA include regulation and coding for histones, ribosomal RNA, and transfer RNA. The nucleoprotein fibers (DNA and histone) of which the chromosome is composed are called **chromatin**. In the past five years, significant advances have taken place in the investigation of the structure of chromatin and nucleosomes (or nu bodies). This subject will be treated later in the chapter.

CHROMOSOMES

As noted in Chapter 1, the chromosomal constitution of each individual is derived equally from mother and father; in the human, 23 chromosomes are contributed by each parent in the form of a gamete (ovum or sperm). The cell formed by fertilization of the ovum by the sperm is the zygote. Each of the 23 paternal chromosomes in the sperm has a homologue in the ovum. Thus, the end result of the fusion of two germ cells (gametes), each with a haploid number of chromosomes, is a diploid cell having 23 homologous pairs of chromosomes.

Chromosomes (*chromos* = color; *soma* = body) are not individually distinguishable except during cell division, at which time they may be seen under the light microscope as rod-like bodies that stain with basic dyes and have a constriction, the centromere where they attach to the

mitotic spindle. Each chromosome has a characteristic length and position of the centromere. Each of the 46 chromosomes is a member of a homologous pair, one member of each pair being received from the mother and one from the father. The members of a pair are called homologues. Twenty-two of the pairs are similar in both males and females and are designated as autosomes. The homologous chromosomes in each pair of autosomes are usually indistinguishable. The chromosomes in the remaining pair are called the sex chromosomes. In the female the two sex chromosomes are similar and are referred to as X chromosomes. In the male there is one X chromosome and a distinctly different chromosome, the Y chromosome.

Figure 2–1 is a photomicrograph of the chromosomes of a single human peripheral blood leukocyte as they appear under the light microscope in metaphase, the stage of cell division during which chromosomes are most readily studied. In Figure 2–2 chromosomes from a normal male have been individually cut out of the photomicrograph and arranged on the basis of size, position of the centromere, and banding pattern. This convention, except for the banding pattern, was established at a meeting of human cytogeneticists in Denver in 1960 and is thus known as the Denver classification. At a similar meeting in London in 1963, it was agreed to use letter designations for the various groups as shown in Figure 2–2. The array of chromosomes in a form suitable for analysis is called a **karyotype.** Further modifications were added at a conference in Chicago in 1966, to code for numerical

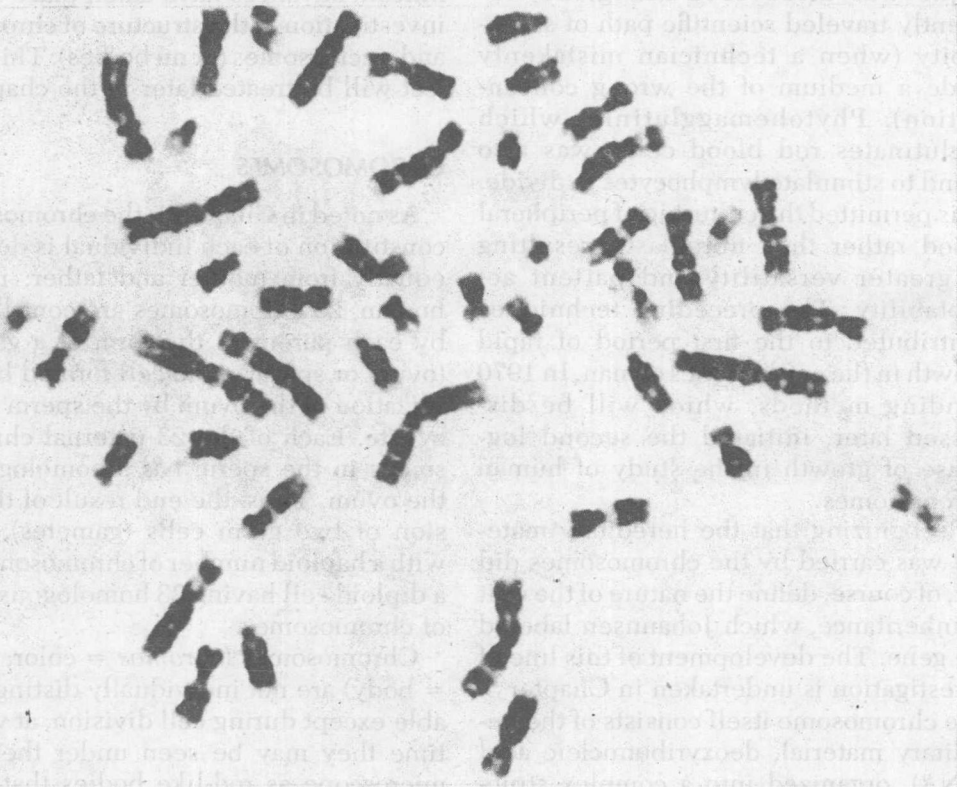

Fig. 2–1. Photomicrograph of human chromosomes in metaphase showing G-banding by the trypsin technique. (Courtesy A. Robinson and D. Peakman.)