Recent Results in Cancer Research

Peo C. Koller
The Role of Chromosomes in Cancer Biology

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With a Foreword by Sir Alexander Haddow

With 42 Figures

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Foreword

For many years Professor KOLLER has possessed an international reputation in the fields of cytogenetics and karyology, both in their fundamental aspects and in their relation to the problems of tumour causation, especially to the role of heterochromatin, and few men have made a greater contribution.

The role of the chromosome complex in carcinogenesis has exerted a natural fascination for many decades, but there can be little doubt of the great advances in knowledge and understanding which have accrued of recent years. Although it is probable that the key event in the inception of particular tumours resides in a delicate molecular rearrangement, and is hence undetectable by conventional microscopical methods, nevertheless a large proportion is accompanied by karyotypic variation and relatively gross changes in chromosomal number, order and arrangement, witness the discovery of the Philadelphia chromosome and its consequences. Further, any such changes in the chromosomal apparatus must inevitably be attended by profound repercussions in the cytoplasm with all that this must mean in protein synthesis and cellular behaviour. It would be idle to pretend that any such problems have finally been solved, and they still contain an element of mystery, as can be seen through a quotation from the address given at the Symposium on "Genetics and Cancer", (Houston, 1959) by DARLINGTON, one of the founders of the discipline of Cytogenetics:

"*Thus there seems no reason to doubt that variations in chromosome numbers occur in tumours not because they matter more, but because they matter less, than elsewhere ... the cell has become less dependent on nuclear balance than in regular development.*"

But it is one function of the present work still further to stimulate our comprehension of these remarkable changes. Although mountains of effort have been expended in attempts to decipher the mode of action of the carcinogenic hydrocarbons, amines and other classes of chemical carcinogen—so far with little success or precision—much progress has come of recent years through study of the reactive alkylating carcinogens. At one stage it almost appeared that the tumour nucleus in such cases might bear, as it were, an imprint of the alkylating carcinogen which induced its appearance. Be that as it may, we are now approaching ever more accurate knowledge of highly specific interactions between these substances and chromosomal DNA-molecules, especially the purines, so bringing about alterations in base sequence and other effects upon the chemical integrity of chromosomal DNA, with all the attendant consequences.

The book describes the molecular organization and function of chromosomes, as well as the consequences of chromosomal aberrations in human development. Not the least impact of cytology on medicine has been of a highly practical kind, and thus the

book also contains accounts of the cellular features of primary tumours and ascitic fluid, and of the cytological actions of radiation and drugs and discusses their relevance to therapy.

On every ground of timeliness and authority, I recommend it warmly, in the certain hope that it must prove of utmost value, at once to those who are already acquainted with its subject matter and to those who are entering a fascinating field.

May 1972 A. HADDOW

Preface

Transformation of a normal cell to a cancer cell is a biological event; it may be referred to as mutation which occurs either at the level of the gene or chromosome. The latter includes the integration of oncogenic viruses into the host cell's DNA. Whatever the mechanism responsible for neoplastic transformation is, it has eventually to affect the genome of the cell. The physical basis of the cancerous cell behaviour is fixed in the molecular organization of the chromosomes and during mitosis it is transmitted through the chromosomes to descendent generations of cells. Abnormal mitosis is a common phenomenon in tumour tissue and has been recognised to be the main cause of chromosomal irregularities which are characteristic features of cancer cells. Already at the turn of the century the possible role of chromosome changes in the aetiology of cancer was being discussed. The discovery of a chromosomal basis of certain pathological syndromes in man e. g. Down's, Klinefelter's, Turner's syndromes, has shown the consequences of anomalous chromosome constitution, and gave new stimulus for similar investigations in tumours.

During the past decade the study of chromosomal aberrations and their significance in the development and progression of tumours became a rapidly expanding branch of cancer research. It has been demonstrated that most malignant growths are of mosaic composition, containing a variety of cell types distinguishable from normal cells and frequently from each other, by their chromosome patterns. The concept of *selective cellular proliferation* has been derived from information gained by studies on karyotypic variation in the cell population of tumours. Chromosome analyses have already brought a clearer understanding of such dynamic processes as cell competition, selection and adaptation, all of which operate within a cancerous growth and have a role in tumour progression and their response to treatment.

I do not intend to present a comprehensive review of the vast literature which has grown up around this subject, full of many paradoxes, discrepancies and descriptions of unexplained phenomena. My aim is to show the value of the information which has been obtained from such studies by considering the chromosomes of cancer cells as a phenotypic characteristic and not solely as the cell components representing the genotype. I hope that colleagues engaged in diverse aspects of cancer research will find the information in the book of interest and help in their own field of study, and those who wish to enter into tumour cytogenetics may find it a useful introduction.

PEO C. KOLLER

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Chapter 1

Chromosome Structure and Function

The concept that chromosomes are essential constituents of cells is nearly a hundred years old. Genetical studies have demonstrated that chromosomes are the carriers of the genes, which determine the hereditary characteristics of the organism. Molecular genetics has given new insights into chromosomal structure and function, its mechanism of replication, the linear sequence of its repeating units which form the genetic code, and the process by which this code is transcribed into a specific protein structure. The chromosomes and their genes are acknowledged to be the biological basis of human variation in health and disease.

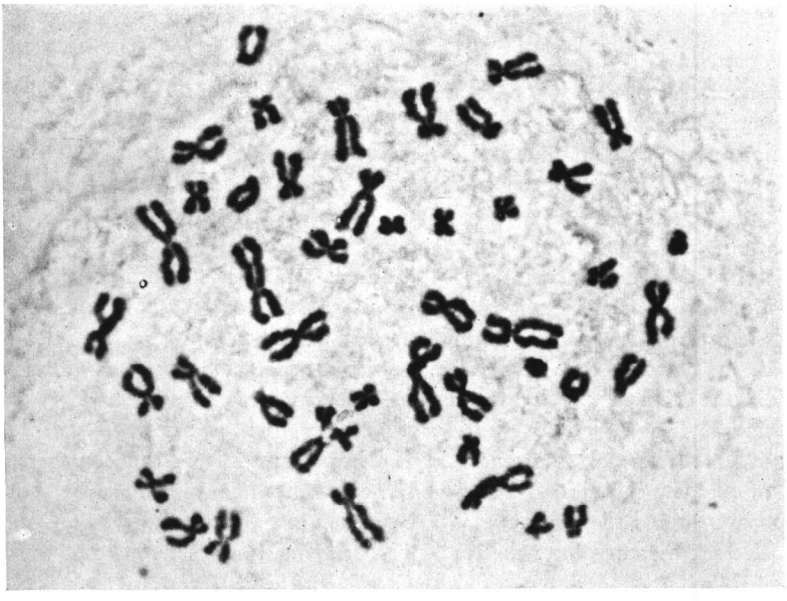

Fig. 1. Metaphase chromosomes of man; each chromosome is composed of two sister chromatids held together at the centromere (By courtesy of Dr. D. T. HUGHES)

The chromosomes can be observed and studied during the metaphase stage of mitosis, when they appear as solid structures, (see Fig. 1).

The core of the chromosome is formed by the double helix of the DNA (deoxyribose nucleic acid) molecule. As well as DNA the chromosomes contain a large amount of basic protein material, which is mostly made up of histones and these

very probably act as suppressors in the regulation of gene expression during cellular differentiation. It has been shown that in the organization of the chromosomes, DNA is the material basis of genetic information and provides the basis for genetic diversity, since on removal of DNA by the enzyme DNA-ase, this genetic instruction is lost.

Chromosome shape depends on the position of the *centromere* which represents the "dynamic" centre, and is responsible for chromosome movement during mitosis. the position of the centromere is indicated by a constriction in the chromosome body. According to its position the chromosome can be acrocentric (telocentric), subtelocentric, submetacentric or metacentric, (see Fig. 2).

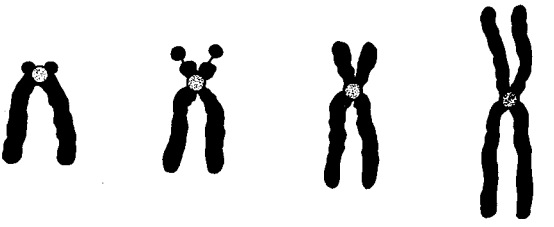

Fig. 2. Diagram illustrates various shapes of human chromosomes: a) acrocentric; b) subtelocentric; c) submetacentric; d) metacentric

The short arm of a subtelocentric chromosome is frequently divided into a small distal segment, which is attached with a long secondary constriction to the proximal region of the chromosome arm. The small segment is called the "satellite". The region at the secondary constriction is often associated with the formation of the *nucleolus.*

The number of the chromosomes is characteristic of the species e. g. man has 46, mouse: 40, rat: 42, Syrian hamster: 44, Chinese hamster: 22. Each cell in the body, with the exception of the germ cells, contains the same chromosome number. Within the cells the chromosomes can be arranged in pairs according to their length and shape. In mammalian cells two chromosomes in the male sex do not match; the shorter is the Y and the longer is the X chromosome. The latter has a matching partner in the female, the constitution of *sex chromosomes* being XX, while in the male it is XY. These chromosomes play an important role in the determination of the sex of the new individual. The other chromosomes not connected with sex determination are termed the *autosomes;* in man there are 22 such pairs, and one pair of XY or XX sex chromosomes; the total number being 46 is referred to as the *diploid* number (2N). The gametes or sex cells (sperm and ovum) contain half the diploid number (haploid = N). Cells with abnormal chromosome numbers are referred to as "*aneuploid*" (or heteroploid). This term indicates only the fact that the chromosome constitution of the cell differs from the diploid cell; it is more precise to refer to the chromosome constitution as hypo-diploid, hyper-diploid etc. thus indicating more precisely the relevant deviation from the diploid constitution. The chromosome complement may be present in multiples of the haploid number, thus there are triploid (3N), tetraploid (4N), hexaploid (6N) etc. cells, all of which are referred to as *polyploid* cells.

According to their length and shape human chromosomes have been classified into definite groups. The autosomal chromosome pairs are arranged in decreasing order of size and numbered from one to 22. They are then divided into seven groups represented by the letters A to G. The X is included in group C with chromosomes 6 to 12, while the Y is placed in group G with chromosomes 21 to 22. The arrangement of metaphase chromosomes into pairs is known as the *karyotype;* this term is applied to the systematized array of the chromosomes from a *single cell* prepared either by drawing or by photography (see Fig. 3).

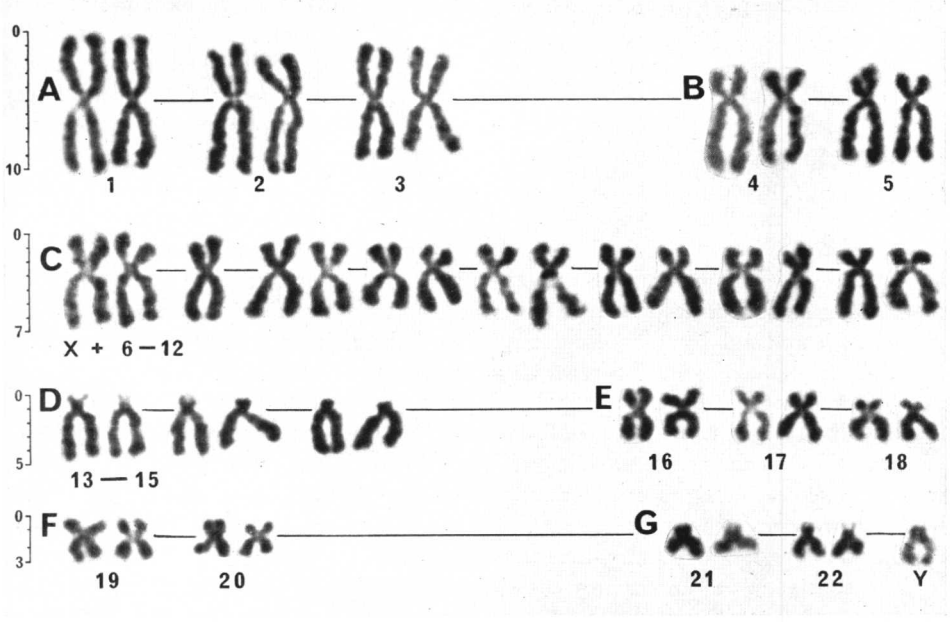

Fig. 3. Karyotype of human chromosomes (male) (By courtesy of Dr. D. T. HUGHES)

The *idiogram* is a schematic representation of the karyotype, which may be based on measurements of chromosomes in *several cells.*

The characterization of individual chromosomes in the human karyotype is very important, and many other features not only those of length and shape are now being used to distinguish chromosomes within groups. Thus a secondary constriction distinguishes one chromosome in group C; a secondary constriction has frequently been seen in the long arm of no. 16 in group E. Satellites have been detected on all three pairs of group D and on both chromosome pairs of group G. From autoradiography using ^{3}H-thymidine, the sequence of DNA replication in various chromosomes or chromosomes regions was determined; differences in labelling patterns are now used to identify individual chromosomes. Tritiated thymidine labelling during the replication of chromosomes reveals the period and duration of DNA synthesis. It was found that the X and Y chromosomes are late in replication in relation to the other two chromosome pairs of group G; chromosome no. 17 terminates DNA synthesis earlier than no. 18, though both are members of group E.

A new approach to the identification of human chromosomes has been made by CASPERSSON and his associates (1970). These investigators found that the highly fluorescent alkylating agent: quinacrine mustard effects discrete, fluorescent labelling of metaphase chromosomes. Regions fluorescing particularly strongly with quinacrine mustard have been demonstrated in chromosomes of 3, 13—15 and Y. The presence in the human chromosomes of specific fluorescent banding patterns as revealed by quinacrine mustard, is unique and reproducible and thus permits identification of particular chromosomes (ROWLEY and BODMER, 1971). Computer analysis was applied to the fluorescence patterns of chromosomes within group C and the eight types within the group have been identified. The method will be most useful for the detection of translocated chromosome regions and their source.

Staining differences in metaphase chromosomes have been observed visually and subsequently measured by microdensitometry. Densely staining regions are usually localised near the centromere and are believed to indicate qualitative alterations in the organisation of the chromosome structure; they are referred to as heterochromatic to distinguish them from the normally staining euchromatic parts. in combination with the fluorescent banding patterns the heterochromatic regions can be used as characteristic features of particular chromosomes (CHERNAY et al., 1971).

The two important parameters usually used in karyotype analyses are the length of the long and short arms of chromosomes. Due to difficulties in the material and procedure employed by different investigators, these measurements vary between five and ten percent, which prevents absolute certainty in the identification of partners of homologous chromosome pairs. Through the use of a partially automatic karyotyping system, GILBERT and MULDAL (1971) were able to compare karyograms of each cell with the combined idiogram for all cells in the sample, and their measurements showed a significantly smaller variance between homologous pairs than had hitherto been reported. Their system seems to offer another valuable method for the characterization of individual chromosomes.

The constancy of chromosome constitution in the cells of the organism corresponds with the stability of the DNA content in the nucleus of body or somatic cells. Mitosis is the process by which cells keep their chromosome constitution constant, it ensures that cells derived by this process receive the same number of chromosomes and the same amount of DNA as contained in the parental cell. Prior to mitosis the chromosomes and their DNA content replicate. The structure of the DNA molecule was clarified by biochemical and X-ray diffraction analysis. The structural organization of DNA is well suited for selfreplication and transcription of genetic information.

DNA is a large molecule, forming a chain of repeated subunits: the nucleotides, composed of three parts: base, sugar and phosphoric acid. The bases are of two types, purines and pyrimidines; the former have a double ring structure, while the latter consist of a single five-membered ring structure. In the nucleotides of the DNA molecule there are two pyrimidine bases: cytosine (C) and thymine (T); and two purine bases: adenine (A) and guanine (G). Many thousand such nucleotides are joined together through sugar-phosphate linkages and form the polynucleotide chain of the giant DNA molecule. According to the model of WATSON and CRICK (1953) the molecule is composed of two polynucleotide chains forming a double helix in which the base to base attachment represents a series of steps. In this model

one purine base pairs with a pyrimidine base: adenine with thymine (A—T) and guanine with cytosine (G—C) (see Fig. 4).

In the DNA molecule the number of possible variations in the sequence of these four bases is limitless, and it is in the sequence that the genetic information is coded. Segments of varying lengths represent the genes, the primary function of which is the "specification" of protein molecules. Proteins are giant molecules in which many

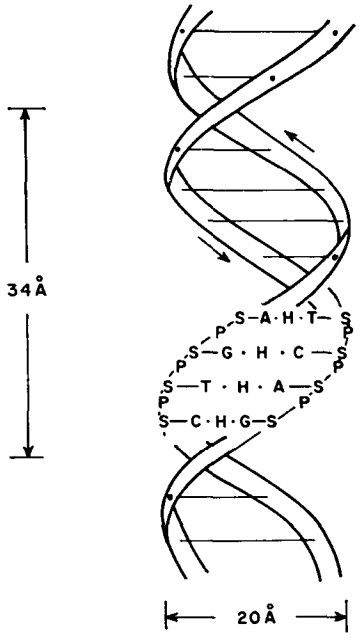

Fig. 4. Watson-Crick model of the DNA molecule: the two sugar-phosphate-sugar chains are held together by hydrogen (H) bonds between their bases, and form a double helix — A: adenine; T: thymine; G: guanine; C: cytosine; S: sugar; P: phosphate group; H: hydrogen bond (After WATSON and CRICK, 1953)

hundreds of amino acids are linked together through peptide bonds, their specific structure and function in cell metabolism depends on the sequence of their amino acid components. Three adjacent nucleotide bases (codon or triplet) in the DNA molecule are required for the selection of a particular amino acid.

The code of the genetic information is embodied in the gene; according to the current concept, the gene is represented by a certain number (600—1500) of purine-pyrimidine bases arranged in a specific linear sequence in the double stranded DNA helix. Recent studies suggest that the chromosome region which is "heterochromatic" is composed of highly repetitive nucleotide sequences. Microbial biochemical genetics provided evidence which showed that in some cases genes coding for amino acids required for one particular protein are situated close together in the chromosome. The function of a gene (or cistron) is that of a template which transcribes the code to a single stranded "messenger" RNA (mRNA-ribose nucleic acid). RNA differs from DNA in having the base uracil (U) substituted for thymine and the sugar ri-

bose in place of deoxyribose. The mRNA molecule leaves the nucleus and in the cytoplasm its base sequence is read by the *ribosome*, on which amino acids are collected. The ribosome is a cytoplasmic subcellular organelle covering the outer surfaces of the three-dimensional network of membranes known as the endoplasmic reticulum. According to the base sequence of the messenger RNA, amino acids are selected and linked together *via* peptide bonds to form polypeptide chains which are then released into the cytoplasm.

The giant DNA molecule with its limitless variation of nucleotide base sequences can store the total genetic information on which the characters and individuality of the organism depends. Although not all genes i. e. all the information coded in DNA, are used at a particular time, cellular metabolism and differentiation are the result of the integration of the whole system *(genome)* as represented by the intact chromosome set. Mitosis is the process by which daughter cells obtain identical genomes. Previous to mitosis the chromosomal DNA replicates, the two parental DNA strands acting as templates on which complementary DNA strands are synthesised. When mistakes occur during the copying of the DNA template, in the process of self-replication, the DNA-repair enzyme corrects the faulty base sequence. It has been observed that if the chromosome structure is altered by physical or chemical agents, or if the genome is either deficient or contains extra chromosome material then this leads to a disturbed genetic equilibrium which affects the behaviour of cell and organism.

Although the structure of DNA which makes up the genetic material of the living organism is now well known, one of the main problems of molecular biology is to find out how the DNA is packed in the chromosomes of higher organisms. The amount of DNA contained in mammalian chromosomes is much larger than is needed to code for all the various proteins. The question is: what is the role of the excess DNA and where is it located? A new theory attempting to answer this question was put forward by CRICK (1971) who suggested that chromosomal DNA falls into two classes: *fibrous*-DNA containing the genes which code for protein, and *globular*-DNA which is located in unpaired regions of the double helix and controls the activity of genes. Just as changes in the genes of fibrous-DNA would result in the impairment of cellular metabolism, so would changes occuring spontaneously or induced in the constituents of globular-DNA. Under this hypothesis malignant behaviour of cells can therefore be attributed to alterations occuring at various levels in the complex organization of the genome.

Summary

Chromosomes are essential constituents of cells; their number and morphology are a characteristic property of the species. The genes are the material basis of heredity and their information is coded in the DNA molecule which forms the backbone of the chromosomes. The structural organization of DNA and its role in protein synthesis have been clarified by molecular biology. The constancy of the chromosome constitution in cells reflects the stability of the DNA content in the nucleus of somatic cells, and this is maintained by the process of mitosis. The chromosomes and their genes are acknowledged to be the biological basis of human variation in health and disease.

Chapter 2

Chromosomal Anomalies as the Cause of Developmental Disorders

Many instances have been found in plants and animals where both abnormal development and the transmission of hereditary characters were associated with chromosome anomalies. BRIDGES (1916) discovered the presence of an additional chromosome in the genome to be responsible for the anomalous transmission of certain characters in the fruitfly Drosophila. He attributed the abnormal chromosome constitution to be the result of non-disjunction of a particular chromosome during gametogenesis. Similarly in Datura plants, the presence of an extra chromosome in the genome produced new morphological varieties. BLAKESLEE (1922) demonstrated that the different appearance or phenotype of the new varieties depended on which particular chromosome of the genome was "extra". Since that time an impressive amount of information concerning chromosome behaviour in plants and animals has been brought together by cytologists showing that the genetic behaviour of an organism can be inferred and predicted through chromosomal studies.

In view of the fact that many developmental abnormalities in plants and animals have been found to be due to chromosome anomalies, several geneticists considered the possibility that certain aberrations of human development could be associated with chromosome abnormalities. Thus HALDANE (1932) suggested that aberrations in human sex differentiation may have a chromosomal basis; and PETTERSEN and BONNIER (1937) discussed the possibility that chromosome anomalies could explain certain types of human intersex. Similarly PENROSE (1939) considered chromosomal irregularity to be the cause of "mongolism" (Down's syndrome), a view well in advance of the cytological studies which, twenty years later, clarified the chromosomal basis of this condition. In 1937 the present author analysing meiotic division in man observed a dicentric anaphase bridge with acentric fragments, and suggested that this might be due to a structural change (inversion) of a chromosome segment (KOLLER, 1937). But all these suggestions met with opposition from other geneticists who believed that the developmental complexities of a human organism could never be determined by a chromosome set so badly disordered as to be visibly different from the normal.

Studies on the chromosomal basis of inherited or congenital anomalies became possible after 1956 when cytological techniques were improved and the exact number of human chromosomes determined. The misconception prevalent for many years that chromosome abnormalities were unlikely to occur in viable persons was dispelled by the discovery of a large number of such chomosome anomalies. Studies

have revealed the association of many congenital abnormalities with particular changes in the chromosome constitution. Human cytogenetics (the study of chromosomes in man) has become of great clinical importance; it is estimated by such studies that in Britain every year nearly 10,000 children are born with chromosome abnormalities, the effects of which range from apparent harmlessness to inevitable death. Every kind of cytogenetic peculiarity previously discovered in plants and animals has now been observed in man. The types of chromosome abnormality that arise naturally or which can be induced experimentally may affect the number or structure of chromosomes, and not infrequently both kinds may be present in the same cell.

1. Numerical Changes

Alterations in the number of chromosomes arise through errors occuring during division, e. g. non-disjunction. lagging of chromosomes, endomitosis, multipolar spindle or failure of spindle formation. When such errors occur during gametogenesis the sperm or ovum will carry an abnormal number of chromosomes and if such gametes become fertilized every cell of the developing individual will have the abnormal chromosome number, provided that the abnormality is viable.

Table 1. Numerical changes and their consequences in man

Chromosome		Syndrome
Number	Constitution	
45	44 + XO	Turner's (female appearance) (1/5000)[a]
47	44 + XXX	Ovarian hyperfunction (mild mental defect) (1/1500)
47	44 + XXY	Klinefelter's syndrome (male appearance) (1/750)
48	44 + XXXX	Mental deficiency
48	44 + XXXY	Klinefelter's syndrome
48	44 + G(21) + XXY	Down's and Klinefelter's S.
49	44 + XXXXY	Mental retardation, skeletal defects, sex anomalies

[a] Incidence of persons in the population born with the syndrome

LEJEUNE et al. (1959) were the first to report an abnormal chromosome number in man. They found 47 chromosomes in the cells of a "mongol" child and suggested the cause to be the presence of an extra chromosome 21 (trisomy-21). Their observations were confirmed within a few months by others, and intensive chromosome studies of many congenital abnormalities were begun. It was soon reported that persons with Klinefelter's syndrome also had a 47 chromosome constitution, the extra chromosome in this case being an X chromosome, (44 + XXY). In Turner's syndrome on the other hand, only 45 chromosomes were found, the missing chromosome being one of the sex chromosomes. The most extensive chromosome alteration was observed in an eight-week old embryo who had a triploid chromosome constitution. Some examples of abnormal chromosome numbers in man and the syndromes they produce are shown in Table 1.

Cytogenetical and clinical studies have shown that the manifestation of a syndrome associated with the same chromosome aberration is not constant or uniform. The effects produced by the excess or loss of particular chromosome can vary; they may be very mild or so severe that they cause death during foetal life.

Table 2 illustrates the complex spectrum of pathological alterations which can be produced by the presence of one additional chromosome in the genome. The wide and variable expression of the syndromes may be attributed to interference by the genes of the extra chromosome with the whole genome. The manifestation of the syndrome can also be influenced by the genetic differences which exist between individuals of the human population.

Table 2. Autosomal trisomy syndromes

Chromosome anomaly	Phenotypic anomalies[a]
D-trisomy (13—15)	Eye defects, deafness, polydactyly, cleft palate, seizures, haemangioma, harelip, anomalous palmar creases, interventricular septal defect, mental retardation
E-trisomy (18)	Failure to thrive, malformed ear, micrognathia, hernia, hypertonicity, defective ossification of sternum, flexion fingers, hip abduction, mental retardation
21-trisomy	Short stature, small round head, protruding fissured tongue, abnormal thyroid function, anomalous dermatoglyphic patterns, immature leukocytes in blood, prevalence to leukaemia in childhood, decreased blood-calcium levels, mental retardation

[a] Compiled from reports of several authors

Trisomy of autosomes is relatively rare and their effects on the individual are so severe that they are usually lethal to the developing embryo as autosomal trisomies not found in life have now been identified in spontaneously aborted foetuses. Theoretically, the genetic imbalance due to the addition of an extra chromosome in the genome can be alleviated by the loss of another chromosome, in which case the chromosome constitution of the cell has been altered but the number of chromosomes remains "normal", though it is referred to as *pseudo-diploid* to distinguish it from the true diploid cell.

2. Structural Changes

Structural alterations are recognised by the new shape and size of chromosomes, or by the irregular pairing behaviour of the homologous chromosomes during gametogenesis; such aberrations do not alter the number of chromosomes in the cell. Most of the reported structural anomalies have been *translocations*, i. e. transfer of a segment from one chromosome to another. Frequently two different chromosomes exchange parts, the phenomenon is referred to as reciprocal translocation or *interchange*. Other structural defects are much less common.

The first case of a structural chromosome anomaly not involving alteration of the chromosome number was described by POLANI and his associates (1960). These investigators found a female "mongol" child who had 46 chromosomes instead of