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HUMAN CHROMOSOMES

E. H. R. FORD

Academic Press London and New York

The Biological Basis of Medicine

edited by
E. Edward Bittar
Department of Physiology
The University of Wisconsin
Madison, Wisconsin, USA
assisted by
Neville Bittar
Department of Medicine
The University of Wisconsin
Madison, Wisconsin, USA

This six volume work provides a logical and complete coverage of human cellular biology and its relationship to medicine, and assumes that cell biology will ultimately unify the basic medical sciences. Adopting an interdisciplinary approach, the editors have collected papers from contributors all over the world. Each topic is covered by leading experts in the field.
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'. . . will be welcomed by doctors and scientists who are concerned to preserve for themselves an intelligent and relevant understanding of modern medicine and as source books of modern knowledge.'
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This is a book for everyone interested in human chromosomes – what they look like, of what they are made, what they are for and how they work. Dr Ford adopts a broad approach, collecting recent observations and offering some original comments on chromosome structure, morphology, identification, and behaviour at mitosis and meiosis, and also discussing chromosomal abnormalities in man and their transmission. As far as possible the work is based on observations and experiments on human material. Special emphasis is laid on meiotic studies, the new fluorescent staining and banding techniques for identifying chromosomes are described, illustrations of karyotypes are given, and the author speculates on the future of the discipline. The final chapter, by Dr J. H. Renwick, gives an account – unobtainable elsewhere – of recent advances in chromosome mapping in man. Over 600 up-to-date references are given.

The work will be of unrivalled value to students, both postgraduate and undergraduate, in medical schools and to research workers in departments of genetics, cytogenetics, cell biology and developmental biology. It will also interest many practising clinicians, particularly obstetricians, paediatricians and those concerned with mental subnormality.

Front/ Electron micrograph of two human chromosomes (a No. 1 and a G group chromosome), fixed and spread as for light microscopy, and stained with uranyl acetate and lead. ×25,000.

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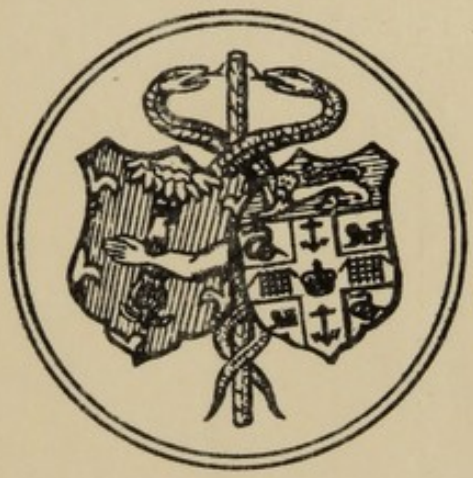

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Human Chromosomes

E. H. R. FORD
Department of Anatomy
University of Cambridge
Cambridge, England

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Preface

This book is intended for anyone who is interested in human chromosomes; in what they look like, of what they are made, what they are for, and how they work. The median reader aimed at is the student of medicine, undergraduate or postgraduate, but I have tried to write as plainly as possible in an attempt to make the book comprehensible to anyone with some background in biology and chemistry. At the same time I hope that more senior research workers will find the book to be a useful compendium of up-to-date information and references.

This is not directly intended to be a textbook of medical cytogenetics; it does not deal specifically with the medical aspects of all chromosomal disorders; there are other books which already do this. Nor is it a comprehensive treatise on human cytogenetics; J. L. Hamerton's great two-volume work "Human Cytogenetics" is likely to remain the standard work on the subject for some years. This book is primarily concerned with the principles underlying the study of human chromosomes, and with trying to give an answer to the question "What may be gained by studying the human chromosomes in relation to such-and-such a problem?". It is true that a good deal of medical cytogenetics has crept in during the course of consideration of such principles; and in the final chapters there is some speculation on the future of human chromosome studies. Although these have not yet brought great dividends in terms of human welfare, I believe that the time when they will be able to do so is fast approaching.

I have tried deliberately as far as possible to confine consideration to the human chromosomes; thus on the one hand there is no discussion of possible mechanisms of cytoplasmic inheritance, or mitochondrial DNA; and on the other hand I have not included much description of work on the genetics and cytogenetics of other forms of life, animals, plants or microorganisms. Much of what is known of human genetics and cytogenetics has come through such studies; but I believe that there is now enough knowledge of human cytogenetics available to allow many problems to be tackled on the evidence from it alone, and I have preferred to leave the comparative

approach to others better qualified to deal with it, except where some topic clearly demands insertion.

The breadth of the field covered, ranging from the ultrastructure of chromosomes through the cytogenetics of human mitosis and meiosis to population cytogenetics, mutagenesis and neoplasia, and chromosome mapping, means inevitably that some topics are covered in greater depth than others. I was uncertain, for example, whether or not to include a section on molecular genetics; but decided that it would be right to do so (even if the approach will seem very elementary to most present day students) for the sake of completeness, and because of its bearing on recently introduced differential staining methods for human chromosomes, which must relate to their physicochemical structure. I have deliberately steered clear of consideration of sex determination, and the problem of intersexuality in man, partly because I have little personal experience in this complex field, and partly because Hamerton's recent book deals with such problems in considerable detail.

The reference list is somewhat like the top of an iceberg; it represents the top of a larger list, which I considered unnecessary to give *in toto*. The list given is rather loaded in favour of recent references, on the grounds that older references are more fruitfully covered by the reading of review articles. But a representative selection has been included of original references to most important topics, and these, together with the books and review articles quoted, will lead the enquiring reader as far as he or she wants to go.

It is a humbling experience to attempt to write a book of this kind; one realizes the insignificance of one's own contribution to the subject, and the immensity of one's indebtedness to the thousands who have toiled all over the world to bring our knowledge to its present state. It would be impossible to read all the relevant contributions which have been made; indeed they are probably being written even now at such a rate that no individual could read them as fast as they are produced. However, as an Englishman, I count myself extremely fortunate that English is now the most used scientific language, and I would like to express my personal gratitude to all those workers who write their papers in English, although it is not their mother tongue. If the reference list displays a certain chauvinism, this is due to the relative availability of journals to me; thus if two equally relevant papers have appeared in the *Lancet* and the *New England Journal of Medicine*, or in *Nature* and *Science, N.Y.* the former is most likely to have been selected, for the reason given above. It is in the nature of scientific research that when a new

technique is devised, it is exploited simultaneously in several different places, each of which will in due course publish comparable findings.

The last chapter, on chromosome mapping, is contributed by Dr J. H. Renwick, to whom I am extremely grateful. I was particularly anxious to include a section on this rapidly expanding subject, but I was incapable of writing an up-to-date account. Dr Renwick very kindly agreed to do this, at short notice and at considerable personal inconvenience, and I believe that his authoritative contribution, with its delightful illustration, rounds off the book in a most valuable way.

I have enjoyed writing this book, and the experience gained thereby has been invaluable. If someone, somewhere, finds it useful, I shall feel adequately rewarded.

November, 1972 E. H. R. FORD
Selwyn College, Cambridge

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I should like to begin by thanking all those who have allowed me the time and afforded me the facilities to write this book. First and foremost my wife, Anna, and my family, for looking after me and helping me while I worked at home; the University of Cambridge and the Master and Fellows of Selwyn College for allowing me nine months' leave of absence; Professor R. J. Harrison (who originally suggested my writing this book) and my colleagues at the Anatomy School and Selwyn College, for arranging for my work to be done in my absence. I should also like in particular to thank my friend and collaborator, David H. M. Woollam, for the many helpful and stimulating discussions we have had together.

Special thanks are due to Raith Overhill, who drew all the original figures, and to Judith Spencer who did much of the typing; also to Ken Thurley, who gave invaluable help in the production of the electron micrographs illustrated, and to Valerie Harrison, Marie Fordham, and Pamela Mees who were severally responsible for the chromosome preparations illustrated.

I am also especially grateful to Dr Jean-Marie Luciani for allowing me to reproduce his beautiful photographs of human meiotic chromosomes, and to Dr F. R. Engmann for letting me reproduce pictures of chromosomal satellites from his Ph.D thesis.

I am also grateful to Drs C. E. Ford and H. M. Clegg for allowing me to quote extensively from their article in the *British Medical Bulletin* 25 (1965), to the late Professor L. S. Penrose and the *Lancet* for permission to reproduce Table 10, to Drs A. T. Sumner, H. J. Evans and R. A. Buckland and *Nature, New Biology* for permission to reproduce Fig. 18, and to the staff of the MRC Clinical and Population Cytogenetics Unit for supplying me with Fig. 17; also to Professor T. Caspersson and his colleagues, and the Academic Press of New York for permission to reproduce Fig. 16 (from *Experimental Cell Research*), and to Dr G. P. Clein for Fig. 15.

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for permission to reproduce his delightful figure of "Otto Soames" (of which he holds the copyright).

E. H. R. FORD

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§ 1 §

Historical Outline

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I. INTRODUCTION

The detailed study of human (and indeed of mammalian) chromosomes began in 1956. Before that year even the number was not known with certainty, and the morphology of individual chromosomes was virtually unknown. The demonstration by Tjio and Levan in 1956 that a preparation could be made which would show each individual human chromosome separately, so that the chromosomes could be accurately counted, and their shape studied, initiated a period of explosive evolution of human cytogenetics which is still continuing.

Human cytogenetics may be considered as a rather late-developing subject within the whole field of genetics. By 1956 the general principles of genetics had been firmly established for a generation at least; and the principles of cytogenetics were also well known, largely through studies on the fruit fly *Drosophila* and on plant chromosomes. These studies had started at the beginning of this century, and by the 1930s they had become highly sophisticated. The existence of this firm foundation, combined with the introduction in the last 20 years of techniques which have revolutionized the whole biological field, and which were not available when the classical studies on genetics and cytogenetics were being carried out, has enabled research carried out since 1956 to yield more information about the human chromosomes and their behaviour than is available for the chromosomes of any species other than *Drosophila* and perhaps a handful of economically important plants (Hamerton, 1971).

This book is about human chromosomes, and discussion of comparative cytogenetics has been deliberately excluded, except where it is essential to understanding. However, much of the knowledge of how human chromosomes function is based on studies on the chromosomes of other species, and particularly of plants. Hypotheses about chromosome function, which are based on the behaviour of the chromosomes of plants or of invertebrates, have been applied to explain the way in which mammalian chromosomes behave; many of them have been tested on human chromosomes, and have been found on the whole to be sound. However, the principle which has been adhered to in this book is that whenever possible the facts are drawn from experiments on mammalian and human material, and unconfirmed hypotheses based on work on other classes and phyla are treated with caution.

II. THE MAINSTREAM OF GENETICS

It is not intended here to give a critical account of the development of the whole subject of genetics; a number of competent histories of this complex subject are available. However it seems desirable to give brief consideration to the history of genetics, with two objects in mind; first, to give a framework of time within which the development of human cytogenetics may be set, and secondly to show briefly how the evolution of the subject has depended on technical advances of every kind.

The basic material of all genetics is the breeding experiment. Such experiments have been carried out from time immemorial, but were only put on a scientific basis from the seventeenth and eighteenth centuries onward. Results can not be completely assessed without the application of mathematical techniques, which were first used with any regularity by nineteenth century workers such as Francis Galton, and the immortal Gregor Mendel. Mendel's work illustrated and described the basic principles of the segregation of inherited characteristics so clearly that it has tended to overshadow that of other experimentalists of his period, even of such men as Charles Darwin.

Mendel's work was published in 1866, but it was not generally known until it was "rediscovered" by Correns, de Vries and others in 1900. Much that had occurred in the intervening period enabled it to be exploited much more rapidly than could have been possible at the

time of its publication; in particular the early development of cytogenetics.

This was a consequence of the improvement of microscopic techniques. The first description of the histology of animal tissues was made in 1827 by J. J. Lister (father of the famous surgeon), using his own compound achromatic microscope with a resolution of 1 μ. Further evolution of microscopy was itself dependent on introduction of satisfactory methods of fixation and staining of tissues, and later of section cutting. These were all introduced during the midpart of the nineteenth century, and by the last quarter of the century satisfactory methods were available. Cell division was first described by Virchow (1858), and he showed that all cells arose from other cells. In 1882 Flemming described dividing cells in the human cornea, and introduced the term "mitosis"; he also defined the framework of the nucleus as "chromatin". The term "chromosome" was introduced by Waldeyer in 1888. By that time Van Beneden had already enunciated his law that these structures were derived equally from the nuclei of two conjugating germ cells, and thus equally from each parent; and Weismann predicted the reduction division of meiosis in 1887.

Thus when the work of Mendel was rediscovered it was only a short time before it was possible to identify the segregating and recombining units of reproduction with the chromosomes, the Sutton-Boveri (Sutton, 1903) hypothesis. This is the seminal hypothesis on which cytogenetics is based. At the same time, the significance of sex chromosomes, as segregating, sex-determining structures, was realized (McClung, 1901).

Once the significance of the chromosomes had been realized (namely that they were visible subunits of the genetic material of the nucleus, in which the segregation of the genetic material could be seen to occur at meiosis), the subject of cytogenetics was vigorously pursued, particularly in two species which were especially favourable for such studies, the fruit fly *Drosophila* and maize (*Zea mays,* Indian corn). *Drosophila* was used for breeding experiments by Castle in the first decade of the twentieth century, proving suitable because of the rapidity with which it reproduced, and the large number of heritable features which could be related to the action of single genes. These features were exploited by Morgan, Bridges, Sturtevant and Muller, and enabled linkage groups on chromosomes to be identified, and chromosome maps to be made indicating the order in which the genes lay on the chromosome. In 1913 Bridges showed that the special behaviour of sex-linked genes was related to

their location on the X chromosome, and that their genetic and cytological behaviour could be directly related. Morgan exploited the cytological observation of meiotic crossing over (Janssens, 1909) to explain further the behaviour of linked genes. The whole concept of genes (a term coined by Bridges) as units of heredity strung along chromosomes like beads on a necklace was built up progressively by this group of workers, and was clearly elaborated by Morgan in his last book, "The Theory of the Gene", published in 1926. But *Drosophila* had still more to offer cytogenetics. In 1933, Painter was able to show that the banding pattern on the giant chromosomes in the salivary glands was related to the gene maps of the chromosomes, and that the bands could actually be considered as representing the linear gene sequence.

Work on maize proceeded in parallel with that on *Drosophila,* and studies on linkage were carried out in parallel with cytological studies on meiotic chromosomes. The linkage studies have been summarized by Emerson *et al.* (1935) and the cytogenetic studies by Rhoades and McClintock (1935). The meiotic chromosomes of maize show, at the pachytene stage, a variety of topographical features, particularly variation in size and distribution of "chromomeres" along their length, which enable each one to be identified individually, and cytological and genetic studies to be related as they have been in *Drosophila.* These studies added greatly to knowledge of the whole process of meiotic segregation and of the mechanism of crossing-over.

The cytological aspects of plant cytogenetics continued to be refined during the 1930s by C. D. Darlington and others; such work as was done on vertebrate chromosomes seemed to indicate that although preparations were harder to make for technical reasons, because of the smaller size and tighter packing of the animal cell as opposed to that of the plant, the general behaviour of the chromosomes both at meiosis and at mitosis paralleled that of plant chromosomes.

By the start of World War II in 1939, the general principles of genetics, and their relation to cytology, were well understood. The techniques used during this period of steady evolution of the subject were mostly already known at the beginning of the century.

III. THE GROWTH OF HUMAN CYTOGENETICS

From the time that chromosomes were recognized as significant structures, attempts were made to count the number of

chromosomes in man. However, the first significantly successful attempt was not made until 1912, when von Winiwarter counted 47 chromosomes in a spermatogonial metaphase and 23 chromosome pairs in a spermatocyte, and concluded that the human chromosome number was 48 in women and 47 in men, and that the sex-determining mechanism was the presence of either one or two X chromosomes. The next significant study was by Painter (1921, 1923) who also worked on testicular material. He observed the small Y chromosome and correctly deduced the XY sex-determining mechanism; counts of chromosomes in spermatogonial mitoses indicated that the number was between 45 and 48. In his first paper he somewhat favoured a diploid number of 46, but in his 1923 paper he decided in favour of 48, and this number was generally accepted and reported in textbooks until the work of Tjio and Levan published in 1956 clearly demonstrated that 46 was the correct number.

Although von Winiwarter adhered to his opinion that there was no Y chromosome, this was finally disproved by Koller (1937) in a study of the behaviour of the sex chromosomes during meiosis. A number of other studies of the human chromosomes were made in the interwar period, mostly on testicular material, but while these mainly supported Painter's conclusions they added little to them. Studies on mitosis in somatic cells were generally rather unenlightening, because of the technical difficulty of obtaining satisfactory preparations, and the whole subject of human cytogenetics remained rather stagnant, while the attention of geneticists was focused on the exciting discoveries which were being made on species more favourable for research. After 20 years' acceptance of a chromosome number of 48 the cautious announcement by Tjio and Levan (1956) that the human diploid chromosome number appeared to be 46 hit the cytogenetic field like a bombshell. Their counts were made on cultures of somatic cells (from fibroblasts of human embryos) and consistently gave the diploid number of 46. Moreover the quality of the preparations (illustrated in the paper) was of a different order from anything which had been seen before. The diploid number of 46 was rapidly confirmed by C. E. Ford and Hamerton (1956) on testicular material from three men, both in spermatogonial metaphases and in spermatocytes (which mostly contained 23 bivalents, or chromosome pairs).

Both these sets of observations were based on material prepared by techniques which themselves involved no materials or methods which had not been available for many years. What was new was the

combination and improvement of several techniques which had been used for different research purposes. The most important of these were the following:

(a) The use of colchicine, a substance extracted from the autumn crocus, which has been used for many years in the treatment of gout; colchicine has the specific effect of arresting cells at mitotic metaphase (by inhibiting formation of the mitotic spindle) and thus allowing many cells at mitotic metaphase to be accumulated in a culture; it has the additional advantage that since the chromosomes contract throughout mitotic prophase and metaphase, arrest at metaphase allows them to contract even more than normally, so that they can be more readily separated from each other and visualized.

(b) Pretreatment of mitotic cells with a hypotonic solution (Hughes, 1952; Hsu, 1952) which causes them to take up fluid and swell, thus facilitating the separation of the chromosomes from each other.

(c) The use of improved squash techniques, derived from techniques devised for plant cells in the 1930s; these in turn are dependent on use of the least hardening fixatives available, either acetic acid or acetic-alcohol.

The papers of Tjio and Levan and of C. E. Ford and Hamerton not only gave the correct human chromosome number; they also gave insight into techniques whereby greatly improved chromosome preparations might be made. Other workers were quick to exploit these techniques, and in two or three years' time a flood of papers on human cytogenetics began to appear which has hardly abated since. Many further technical improvements were published, particularly in relation to methods of culturing cells from various sources, and in methods of handling these cultures. These will be discussed later (Chapter 4) and only one further technical innovation will be mentioned here; this is the method of culturing lymphocytes from human blood, first described by Moorhead *et al.* (1960) which has offered a method of obtaining human chromosome preparations more easily and quickly than any other which is generally available.

IV. FACTORS INFLUENCING THE DEVELOPMENT OF HUMAN CYTOGENETICS

The growth period of human cytogenetics has come many years later than the period when the classical fundamental discoveries of

cytogenetics were made, mainly on plant and invertebrate material. During the intervening years many profound advances have been made in the whole biological field, both in knowledge and in technology. These have enormously widened the scope of genetics, and have enabled cytogenetics to be studied in much greater depth, even to the molecular level.

Probably the most important of these advances is the development of the new field of molecular biology. Advances in both physics and chemistry have afforded methods for the analysis of the most complex biological molecules by such techniques as X-ray crystallography and the chromatographic analysis of protein molecules, and these achieved their climax in the analysis of the structure of DNA by Watson and Crick in 1953. The fundamental genetic material in the chromosomes has thus been identified, and although there is still much to learn about the way in which it is packed into chromosomes, replicates itself, and exerts its genetic effects, there can be no doubt that this discovery has revolutionized the study of cytogenetics. Like other fields in genetics, molecular biology has grown from small beginnings to a subject of vast size and momentum. Just as knowledge of the structure of the nucleic acids has grown, so a much clearer understanding has been formed of the way in which they exert their genetic functions; beginning with the pioneer work of Garrod (1899, 1909) on "inborn errors of metabolism", the concept that one gene was responsible for the production of one enzyme (or protein) was gradually built up through the work of Beadle, Tatum and others on simple organisms such as the breadmould *Neurospora* (reviewed in Beadle, 1959). And research in molecular biology since 1953 has clarified the relationship between "genes" and chromosomes, and shown how a "gene" is a complex structure, which can be broken down into subunits, and which can no longer be considered as a discrete entity.

Two important technical innovations have, in particular, enabled the new knowledge of molecular biology to be related to cytology; one is the introduction of the technique of autoradiography, by means of which radioactive "labelling" atoms can be incorporated into newly synthesized molecules of nucleic acid or protein and thus enable the position and speed of synthesis of these to be directly visualized; and the other is the introduction of the electron microscope into biological work (effectively in the 1950s); this has the power to resolve biological macromolecules, and thus to make it possible to relate structure at molecular level to what can be seen with a conventional light microscope.

In addition to these powerful new techniques, knowledge of the structure of nucleic acids, and of the fact that they can be chemically altered (denatured) by specific treatments, has offered the possibility of differential histochemical staining of nucleic acids of varying chemical structure, and this in turn is beginning to enable the identification of individual chromosomes to be made much more effectively. This advance has great implications for the future of linkage studies and chromosome mapping in man; hitherto, advances in these subjects have been severely hindered by the large number of chromosomes found in man (in comparison with, for example, *Drosophila* and maize), and the impossibility of individual identification of each one, let alone the identification of visible structural landmarks within chromosomes which might be used like the banding of *Drosophila* chromosomes or the chromomeres in the pachytene chromosomes of maize in the relating of visible and genetic structure.

The human species is good for genetic studies in some ways, and bad in others. Its good points are the size of the population available for study, the very detailed knowledge available of the physical and chemical structure of the human body, and of its variability and the ready availability of clinical material. Bad points to set against these are the long generation time, the fact that experimental mating is not possible, and (from the cytogeneticist's aspect) the large number of chromosomes, many of which are morphologically indistinguishable. However this last difficulty is being rapidly overcome by the use of the new techniques which have been mentioned, and it now seems likely that in the next generation the available knowledge of human genetics and cytogenetics will easily surpass that available for any other species of plant or animal.

§ 2 §

Mitosis and the Human Mitotic Chromosomes

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I. INTRODUCTION

Chromosomes are dark-staining structures which are visible in dividing cells. They are not normally visible to the microscope in cells which are not dividing. They are the means by which the genetic material of a cell, contained in the nucleus, is transferred to two daughter cells in equal amount when the cell divides. They were first described by Flemming in 1882, and when the work of Mendel was rediscovered at the beginning of the twentieth century it was quickly realized (as has been described in the last chapter) that they represented the structural vehicle for the segregation and assortment of the genes, the hypothetical structures then postulated as the units of genetic information.

Chromosomes are visible at *mitosis*, the division of ordinary somatic (body) cells, and at *meiosis*, the specialized division of the reproductive cells, which involves a halving of the chromosome complement of these cells prior to fertilization, the *reduction division*. The morphological appearance of meiotic chromosomes is somewhat different from that of mitotic chromosomes.

II. MITOSIS AND THE MITOTIC CYCLE

When a cell is not dividing it is said to be at the stage of *interphase*. Mitotic cell division is divided into four stages, *prophase*, *metaphase*, *anaphase* and *telophase* (Fig. 1). These stages of the so-called *mitotic cycle* will be considered one by one.

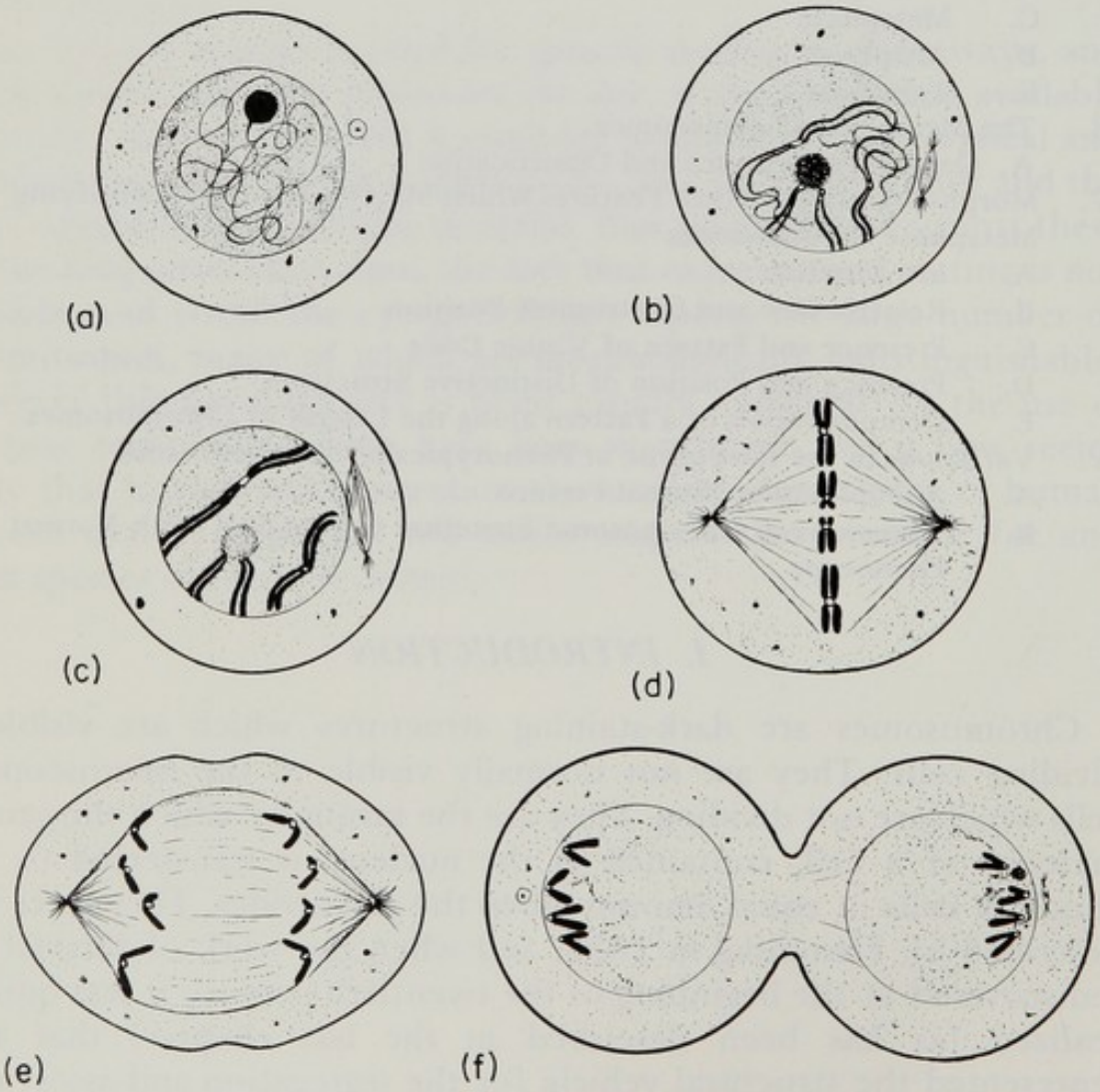


Fig. 1. Mitosis (simplified; only two pairs of chromosomes are depicted). a. Early prophase. b. Mid-prophase. c. Late prophase (prometaphase). d. Metaphase. e. Anaphase. f. Telophase.

A. Interphase

The chromosomes of the interphase cell are not normally visible to the light microscope, but their presence can be demonstrated by techniques which use the higher resolution of the electron microscope. During interphase the chromosomes are active in RNA synthesis. At a certain, rather fixed time before the cell next divides they also replicate themselves and thus double the amount of chromosomal material in the nucleus. The time when they are doing this is known as the S (synthetic) period. The period between the end of the previous cell division and the beginning of the S period is known as the G_1 period, and the interval between the end of the S period and the beginning of the next cell division is called the G_2 period. The length of the S period and the ensuing G_2 period are rather constant for any particular type of cell; thus for human lymphocytes or fibroblasts in culture the S period lasts about 7 hours and the G_2 period 4 to 6 hours. Figure 2 summarizes the stages of

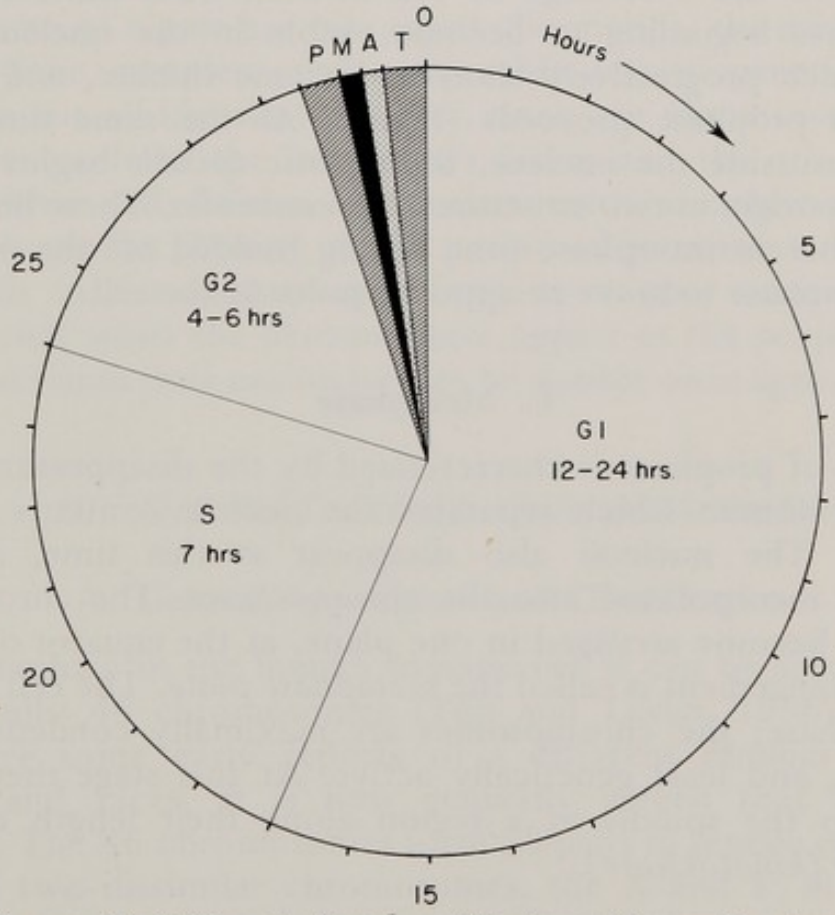


Fig. 2. Diagram of mitotic cycle (based on behaviour of human lymphocytes *in vitro*). P = prophase, M = metaphase, A = anaphase, T = telophase. G_1 = period between end of cell division and beginning of chromosome replication. S = synthetic period during which chromosomes replicate. G_2 = period between end of replication and start of next cell division. All these periods except the G_1 period are fairly constant in duration.

the mitotic cycle in diagrammatic form, for these cells. Much less is known about the duration of the different periods in other types of cell, but there is certainly variation between tissues.

The only structures visible to light microscopy in the interphase nucleus when normal staining methods are used are *chromatin* and the *nucleolus*. Chromatin is a darkly-staining network, without obvious structure, but more condensed in some parts of the nucleus than in others. It sometimes forms discrete highly-condensed regions which have been called *chromocentres*. Chromatin represents what can be seen of the chromosomes at interphase, and has the same chemical structure; it is composed of deoxyribonucleic acid (DNA), ribonucleic acid (RNA) and protein. The *nucleoli*, variable in number but usually between 1 and 4, are rounded, darkly-staining bodies which are mainly composed of RNA.

B. Prophase

Prophase is the first stage of cell division. It is initiated by the chromosomes beginning to become visible in the nucleus as fine threads which progressively shorten, become thicker, and so more obvious, as prophase proceeds (Fig. 1). At the same time, in the cytoplasm outside the nucleus, the *mitotic spindle* begins to form. This has its origin in two structures, the *centrioles*. These lie adjacent to each other in interphase (one having budded off the other) but begin at prophase to move to opposite poles of the cell.

C. Metaphase

The end of prophase is characterized by the disappearance of the *nuclear membrane* which separates the nuclear contents from the cytoplasm. The nucleoli also disappear at this time, and their material is incorporated into the chromosomes. The chromosomes themselves become arranged in one plane, at the equator of the cell, and this arrangement is called the *metaphase plate*. The cell is then at full metaphase; the chromosomes are maximally condensed, most easily seen, and least genetically active. At this stage they become attached to the spindle at a region along their length called the *centromere (kinetochore)*.

D. Anaphase

The next stage of cell division, *anaphase*, begins when the centromere of each chromosome divides longitudinally, so that the

two halves of each chromosome, the *chromatids,* are completely separate from each other. The two sets of chromatids then begin to move away from each other fairly rapidly, under the influence of the spindle fibres, to opposite poles of the cell. The actual mechanism of this movement is still poorly understood. When the sets of chromatids have reached the opposite poles of the cell, anaphase is completed, and the next stage, *telophase,* begins.

E. Telophase

At telophase, the last stage of cell division, the cytoplasm undergoes cleavage to form two completely separate daughter cells. The chromosomes undergo in reverse the process through which they passed at prophase; they gradually elongate and become less distinct, while the nuclear membrane is reformed around them and the nucleoli reappear. The stage ends, and cell division is complete, when the cell has fully reassumed its interphase appearance.

Since the metaphase chromosomes exist in pairs, and each chromosome of a pair has two equal chromatids, the metaphase cell contains four complete and comparable sets of genetic material. These are not all identical however, since one chromosome of each pair has come from the individual's father and the other from the mother. After cell division the nucleus contains only two sets, one from each parent. During the S period of interphase each of the chromatids in the nucleus manufactures another one identical to itself, so that when the chromosomes appear at the prophase of the next cell division they can be seen to be double once again.

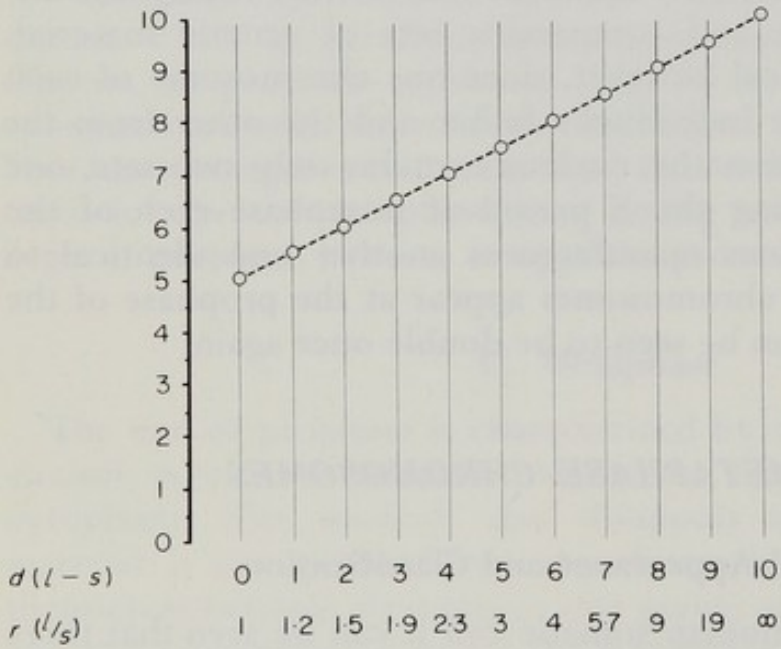
III. THE METAPHASE CHROMOSOMES

A. General Appearance and Classification

At metaphase in the human somatic cell it can be seen that there are normally 46 chromosomes (Tjio and Levan, 1956). Although there were some early reports of a different number in certain subjects and races, it is now generally agreed that these were mistaken. The 46 chromosomes form 23 pairs in female cells, and 22 pairs and two dissimilar chromosomes, the X and Y, in male cells. This number, 46, is referred to as the *diploid* chromosome number. The gametes formed after the reduction division of meiosis have only half this number (22 + X or 22 + Y) and this is referred to as the *haploid* number. Multiples of the haploid number other than diploid are sometimes found and are generally referred to as *polyploid,* or

more specifically as *triploid* (thrice the haploid number, 69), *tetraploid* (four times the haploid number, 92) and so on. Another term in common usage is *aneuploid*, meaning a number which is not an exact multiple of the haploid number (not euploid), and different aneuploidys may be referred to as *hyper-* or *hypo-* (above or below) a particular "ploidy"; thus a chromosome number of 48 might be called "hyperdiploid", or a number of 88 as "hypotetraploid".

Each of the metaphase chromosomes consists of two equal halves, the *chromatids*, which lie alongside each other, and are joined only at the centromere, which appears as a single undivided region of the chromosome, paler-staining than the rest, and constricted (an early term for the centromere was the *primary constriction*). If the centromere is near the middle of the chromosome the latter is called *metacentric*; if somewhat to one side of the middle, *submetacentric*; if near the end, *acrocentric*, and if terminal, *telocentric*. No human chromosomes are normally telocentric, but telocentric chromosomes are found in many animals (e.g. the mouse). Levan *et al.* (1964) have quantified this terminology by a method which is summarized in



Term	Location	*d* value	*r* value
M	Median point	0·0	1·0
m	Median region	0–2·5	1·0–1·7
sm	Submedian region	2·5–5·0	1·7–3·0
st	Subterminal region	5·0–7·5	3·0–7·0
t	Terminal region	7·5–10·0	7·0–∞
T	Terminal point	10·0	∞

Fig. 3. Nomenclature for centromere position (Levan *et al.*, 1964). The chromosome is divided into ten equal parts. The small circles represent the centromere. l = length of long arm in tenths of total chromosome length. s = length of short arm in similar units. d value = $l - s$. r value = l/s.

The centromere positions of the human chromosomes using this system are:

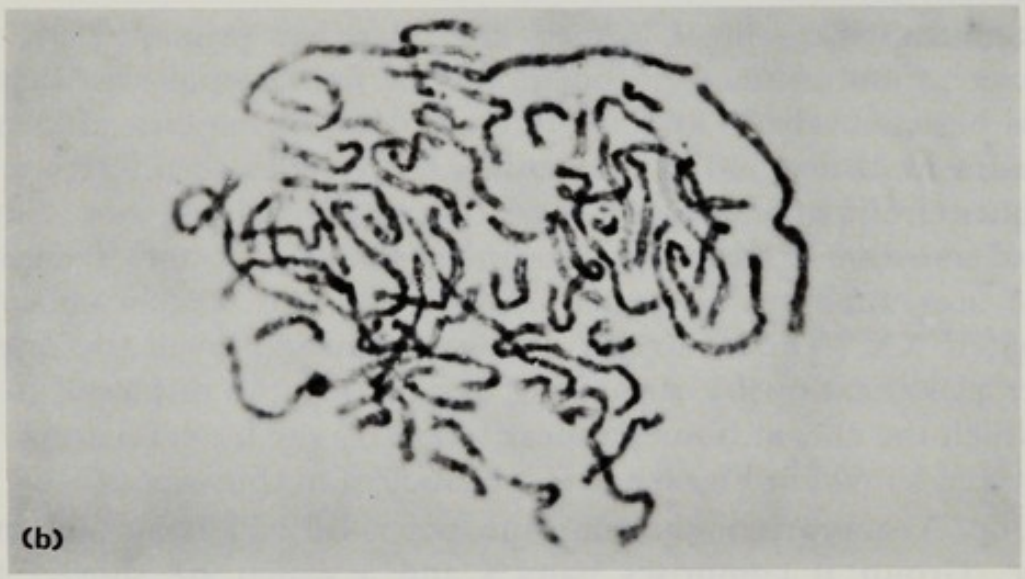
Nos 1, 2, 3	m	17-18	sm
4,5,6-12,X	sm	19-20	m
13-15	st	21-22,Y	st
16	m		

Fig. 3. Using this classification there can be no ambiguity over the description of the centromere position in any particular chromosome.

The appearance of the normal human metaphase chromosomes is shown in Figs 4 and 5, which give an impression of how the chromosomes shorten and thicken as cell division proceeds. For detailed study it is usual to select a cell at full metaphase, photograph the chromosomes, cut them out and arrange them as shown in Figs 6 and 7. Such an arrangement is called a *karyotype.* A related word, *idiogram* is used for a diagram of such an arrangement, as opposed to an actual photograph or camera lucida drawing of the chromosomes.

The metaphase chromosomes are classified primarily on two criteria, size and centromere position. The first formal classification on this basis was made by a conference of cytogeneticists at Denver, Colorado, in 1960, and is thus called the Denver classification. All subsequent refinements of classification are based on this. The 22 pairs of *autosomes* (chromosomes other than the X and Y chromosomes) are numbered in order of size, and the sex chromosomes designated XX (female) or XY (male). Patau improved the original Denver classification by suggesting designating the different groups into which the chromosomes appear to fall by the letters A to G. The karyotypes shown in Figs 6 and 7 are labelled in this way.

Group A comprises chromosome pairs 1-3, all large and metacentric. Group B comprises pairs 4 and 5 which are shorter than group A and are submetacentric. Group C includes pairs 6-12 and the X chromosome(s). These are all submetacentric and broadly indistinguishable, except that there is a gradation of size from largest to smallest. Group D contains pairs 13-15, all acrocentric and morphologically similar. Group E contains pairs 16-18. Pair 16 is metacentric and can be distinguished from pairs 17 and 18 which are submetacentric and similar to each other. Group F comprises two small metacentric pairs, 19 and 20, which cannot be distinguished from each other. Group G contains two small pairs of acrocentric chromosomes, 21 and 22, which cannot normally be distinguished, although there are very slight differences in size and morphology. The X chromosome, as already mentioned, cannot normally be distinguished from other chromosomes of the C group. The Y chromosome resembles the chromosomes of group G, but can usually be distinguished from them on morphological grounds (see later); it is also more variable in size.



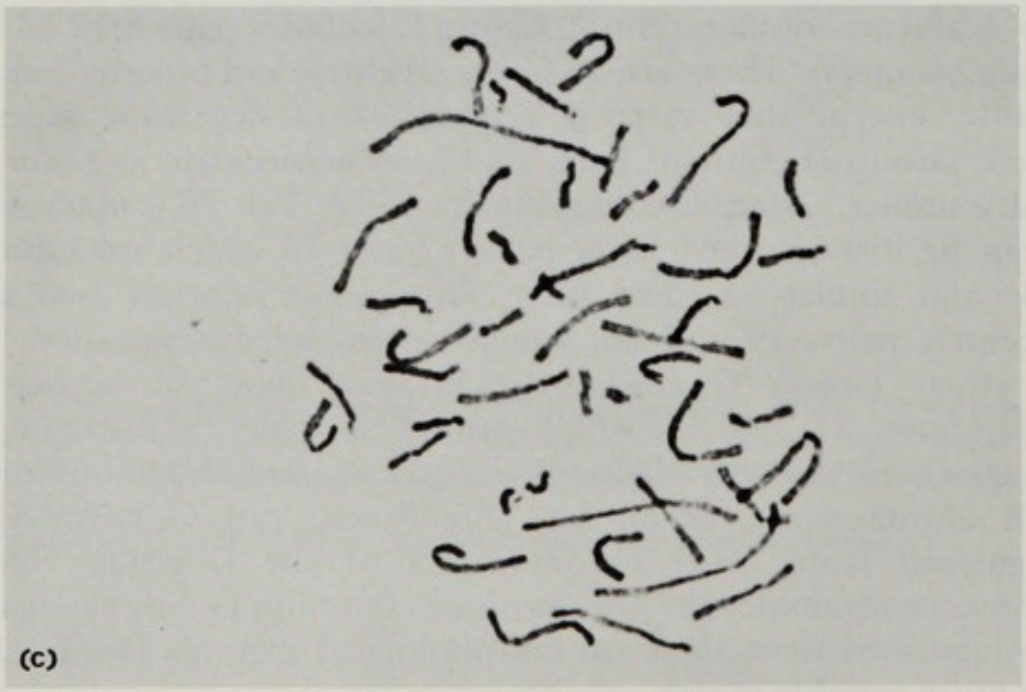


Fig. 4(a-c) above and Fig. 5(d-e) opposite. Human chromosome preparations photographed at the same magnification to show the progressive shortening between late prophase

and full metaphase. a,b—late prophase. c,d—early metaphase. e,f—full metaphase (in f, some chromatids have separated at the centromere, indicating impending anaphase). ×1000.

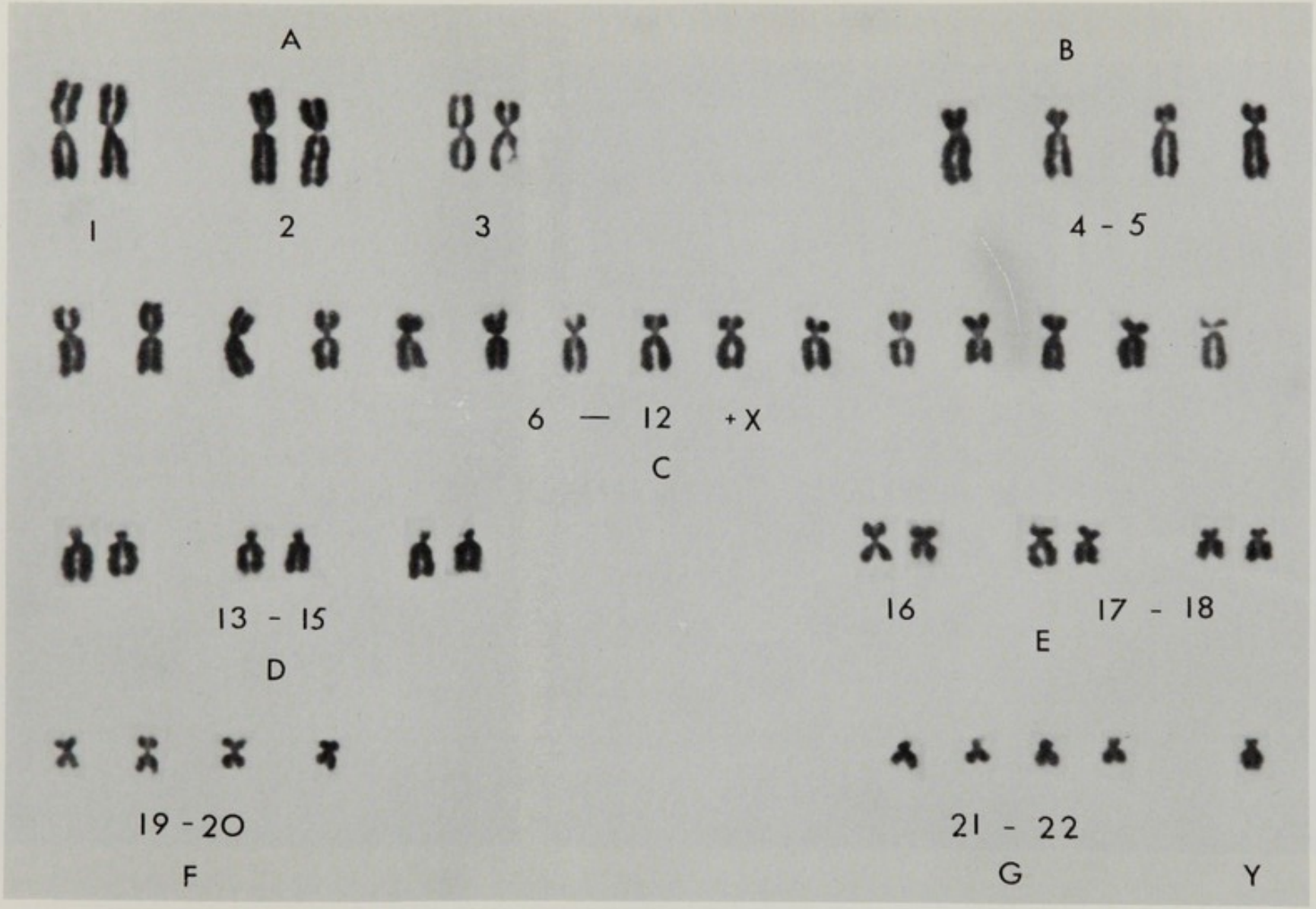


Fig. 6. Normal male karyotype. Chromosome pairs are numbered according to the Denver classification (but no attempt has been made to specify pairs 4-5, 6-12 and X, 13-15, 17-18, 19-20 and 21-22 individually). The capital letters give the Patau groups, and chromosomes X and Y.

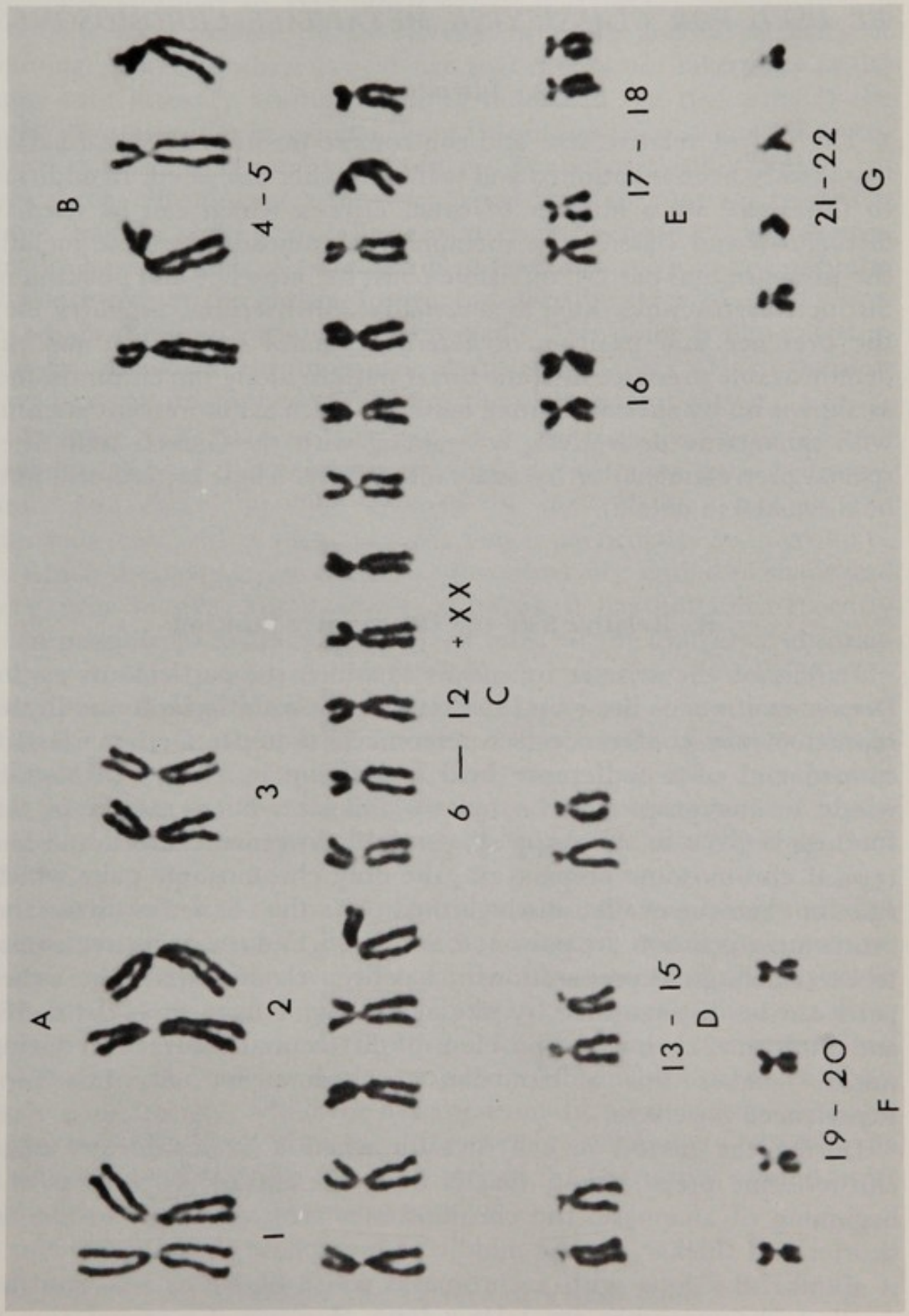


Fig. 7. Normal female karyotype (arrangement as in Fig. 6).

IV. MORPHOLOGICAL AND OTHER FEATURES WHICH MAY BE USED FOR CLASSIFYING METAPHASE CHROMOSOMES

A. Introduction

The use of relative size and centromere position in classification has already been mentioned and will be further discussed. In addition to this there are a number of other criteria which can be used to distinguish and classify the metaphase chromosomes; these include the presence and pattern of visible coils; the presence and position of distinctive structures such as *secondary constrictions, satellites* etc.; the presence and position of *heterochromatin* (see later); and the demonstrable presence of some linear pattern along the chromosome, as shown up by special staining methods (such as fluorescent staining with quinacrine derivatives, or staining with the Giemsa stain after special pretreatment) or by autoradiography. These criteria will now be discussed in detail.

B. Relative Size and Centromere Position

Details of the criteria by means of which the participants at the Denver conference drew up their classification are to be found in the reports of the conference (see reference list under Denver Classification) and of a conference held in London in 1963. Full details would be inappropriate in a text of this size, but a resume of the findings is given in the Appendix, p. 342. In general terms, and in a typical chromosome preparation, the only chromosome pairs which can be unequivocally distinguished on the basis of size and centromere position are pairs 1, 2 and 3, 16, and the Y chromosome. In extremely good preparations it has been claimed that some other pairs can be distinguished by size alone (e.g. 4 from 5, 17 from 18, and 21 from 22), but the problem of differential contraction during mitosis makes this a hazardous procedure for any but very experienced observers.

During the period in cell division when it is possible to make chromosome preparations, that is from the end of prophase to the beginning of anaphase, the chromosomes are continually becoming shorter and thicker. At the middle of metaphase chromosome No. 1 is about 7-8 μ long with a chromatid width of 1 μ or less, and the smallest chromosomes are 1-1½ μ long. However if a preparation is made in very early metaphase ("prometaphase") the lengths may be twice as great; and in a preparation made very late, particularly if

anaphase has been prevented by the use of colchicine (see later) the chromosomes may be very much shorter and thicker. Contraction in length is accompanied by an increase in width and in intensity of staining. Moreover there is evidence that it does not take place at the same rate in every chromosome, or indeed in the two arms of the same chromosome. In general, long chromosomes contract relatively more than short chromosomes during metaphase; and similarly, in any given chromosome, the long arms tend to contract relatively more than the short arms. Thus any attempt to classify chromosomes on the basis of their relative size must take into account not only the absolute size of the chromosomes, but also the stage of cell division at which the preparation has been made. This point is illustrated in Fig. 8a, in which chromosomes at different stages of cell division have been photographed together at the same magnification.

Despite this, it can be said that the relative sizes of the chromosomes of different Patau groups do not alter during contraction; but there may be changes in the relative sizes of the chromosomes within those groups. This is particularly so in group C, in which there are many chromosomes which are similar in shape and very near in size. For example, although it has not until recently been possible to distinguish between most of the individual chromosomes in this group, the inactive X chromosome (see p. 38) can clearly be distinguished by autoradiography; and it has been shown that in early metaphase it is one of the largest chromosomes in group C, but that it contracts relatively more than other members of the group, so that by the middle or end of metaphase it is in the middle or even the lower part of the group by size (Bishop *et al.*, 1965).

This differential contraction of chromosomes is probably related partly to the amount of heterochromatin (*q.v.*) which they contain. Smith (1965) has shown that in the chromosomes of ladybirds, which have one arm which is largely heterochromatic, the arms show strong differential contraction after prolonged exposure to colchicine. The euchromatic (non-heterochromatic) arms contract early and strongly, while the heterochromatic arms contract late and little. It is also likely that the difference in morphology between the Y chromosome and the chromosomes of the G group is related to its probable high heterochromatin content.

C. Presence and Pattern of Visible Coils

Sometimes the chromosomes in standard preparations can be seen to show very clear helical coiling (Fig. 8b). Ohnuki (1965) has

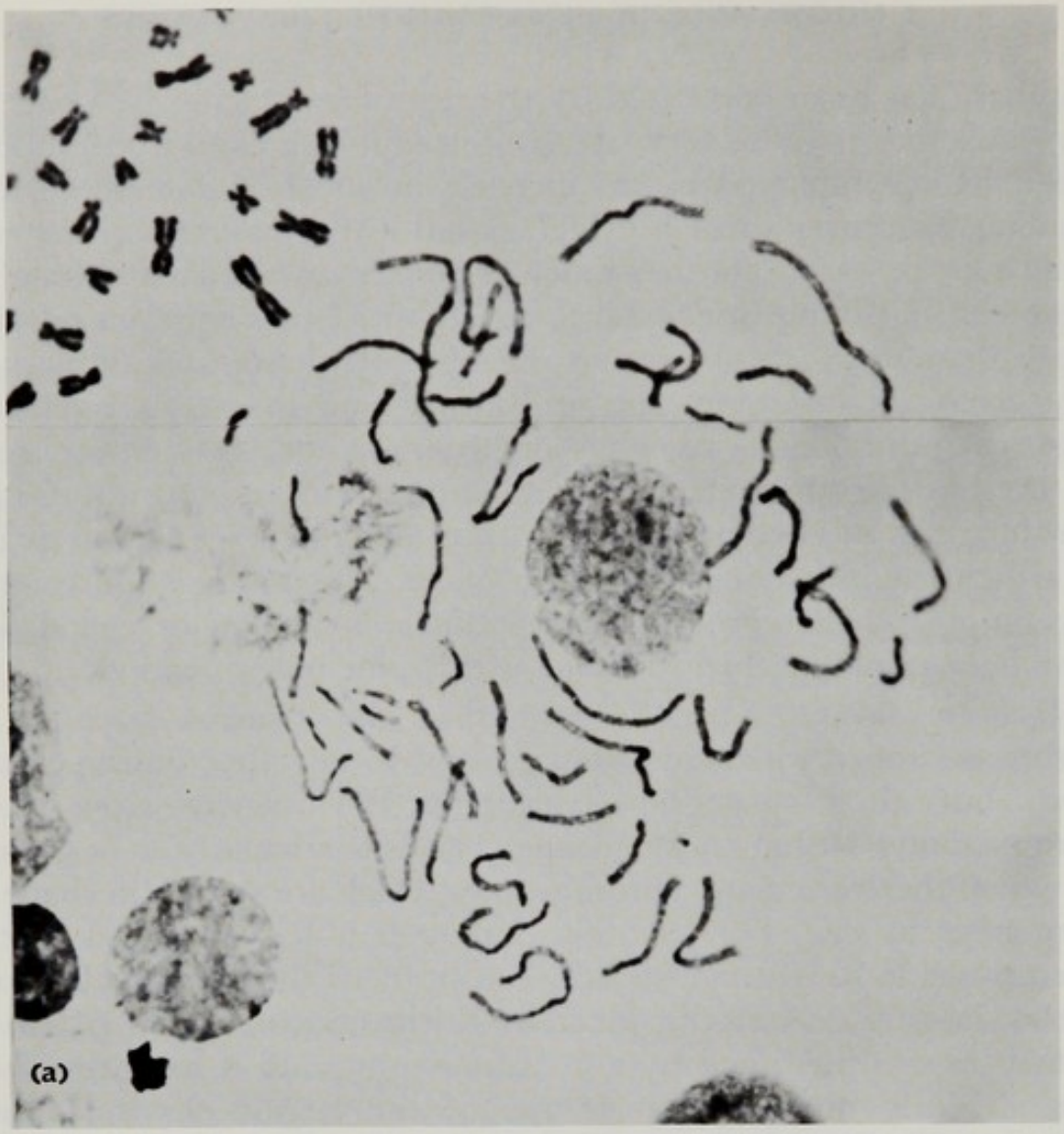


Fig. 8(a). Chromosomes of two cells at prometaphase (bottom right) and full metaphase (top left) to show the degree of contraction occurring between these stages. Some interphase nuclei are also visible. ×1200. (b) Metaphase chromosomes showing helical coiling. ×1200.

described ways in which this coiling can be enhanced, and has published very beautiful photographs of the coiling so produced, suggesting that the morphology of the coils might be an additional aid to chromosome classification (by, for example, making counts of the number of gyres). However while such coiling is a constant feature in some plant chromosomes, it is not sufficiently easily induced in human chromosomes to serve as a practicable method of chromosome identification, nor indeed has it been possible to show that gyre number is constant for a particular chromosome; it may vary with the state of contraction. So that while this coiling is of theoretical interest inasmuch as it probably relates to the mechanism of contraction of chromosomes during metaphase, it does not at present appear that it is likely to be useful for identification.

D. Presence and Position of Distinctive Structures

1. Secondary Constrictions

The *primary constriction* is, as previously mentioned, an old name for the centromere. The centromere is a region which appears constricted and which stains little, if at all, with any of the dyes which stain chromatin. While it has in the past been suggested that the constriction is apparent rather than real (due to lack of stainability) evidence from electron microscopy (including negative staining) indicates that it is a true constriction and that the lack of staining relates to the paucity of chromosomal material present there (E. Ford *et al.*, 1968). A number of the chromosomes show constricted areas in their arms in certain preparations and in certain individuals, and these are called *secondary constrictions.* The positions and relative frequencies of appearance of these have been described by Ferguson-Smith *et al.* (1962) and are shown in Fig. 9. Palmer and Funderburk (1965) have also produced figures for the frequency of appearance of secondary constrictions, and broadly confirm the findings of Ferguson-Smith *et al.* Like the centromere, the secondary constrictions appear to be true constricted regions where the chromosomal material is sparse and despiralized.

Unfortunately for the cytogeneticist the secondary constrictions are not constantly present, and when they can be seen it is often on only one chromosome of a pair. Various techniques have been employed to enhance their visibility such as modification of fixation and spreading techniques (Saksela and Moorhead, 1962), use of calcium-free culture medium (Sasaki and Makino, 1963), cold

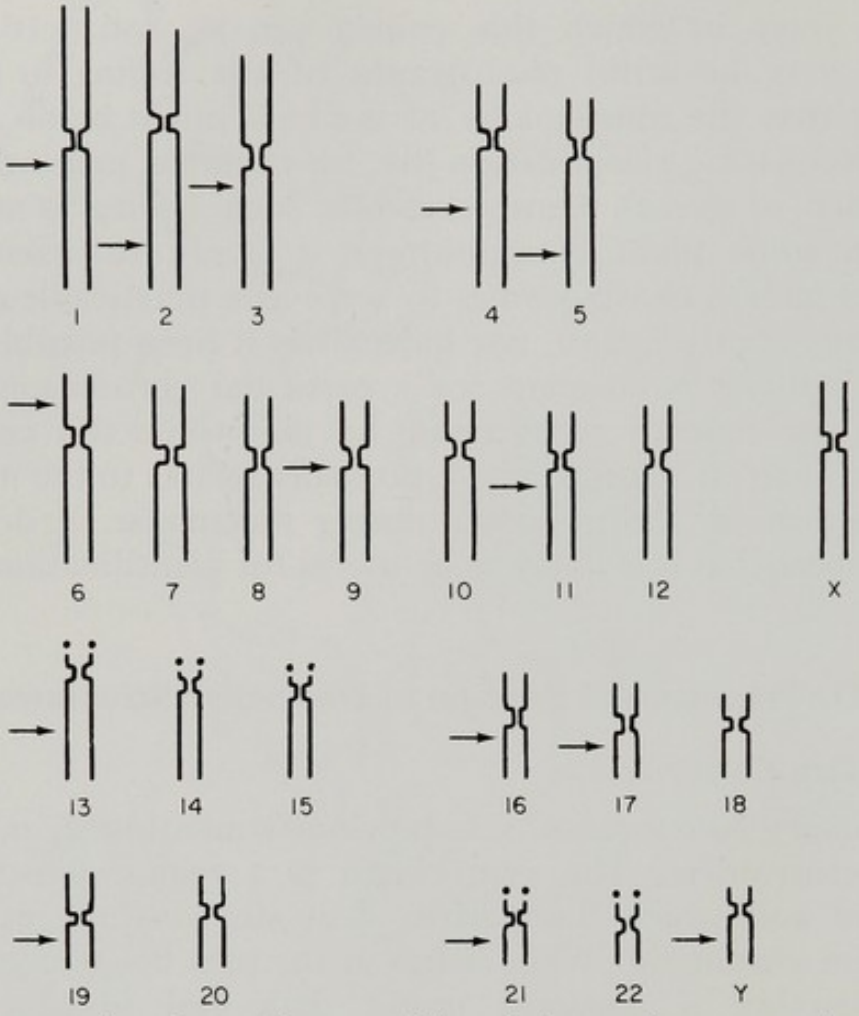


Fig. 9. Common sites of secondary constrictions in human chromosomes (arrowed), after Ferguson-Smith *et al.* (1962).

treatment (Hampel and Levan, 1964), culture in presence of 5-bromodeoxyuridine, a thymidine analogue which inhibits DNA synthesis (Palmer and Funderburk, 1965), and so on, but in general these techniques, while enhancing the appearance of the most obvious constrictions present (such as those on chromosomes 1, 3, 9, 16 and Y) do not invariably show them up as features of those chromosomes. For example, the secondary constriction which is most often seen (apart from those related to the satellites on the D and G group chromosomes, which will be discussed later) is probably that on chromosome C 9, on the long arm close to the centromere. While this is often present on both members of this chromosome pair, it is also often seen only on one member of the pair. This suggests that the constriction may be a structural feature of the chromosome, but one for which chromosome C 9 is polymorphic. There is evidence from the constrictions associated with the chromosomal satellites (see later) that constrictions may be inherited as structural features, which segregate in Mendelian fashion. If this is

so their main importance to the cytogeneticist may be as markers, which can show whether the chromosome in question is paternal or maternal in origin. Indeed a rather exaggerated constriction (or despiralized area) in chromosome No. 1 of occasional individuals has been used as a marker in this way, to demonstrate the probable localization of the Duffy blood group locus to chromosome No. 1 (Donahue *et al.*, 1968).

2. Satellites

Certain of the ten chromosomes of groups D and G (the acrocentric chromosomes) may show a separate small mass of chromatin at the end of their short arms, separated from the rest of the short arm by a marked secondary constriction. These structures have been termed "*satellites*" (Figs 10-12). It is doubtful whether

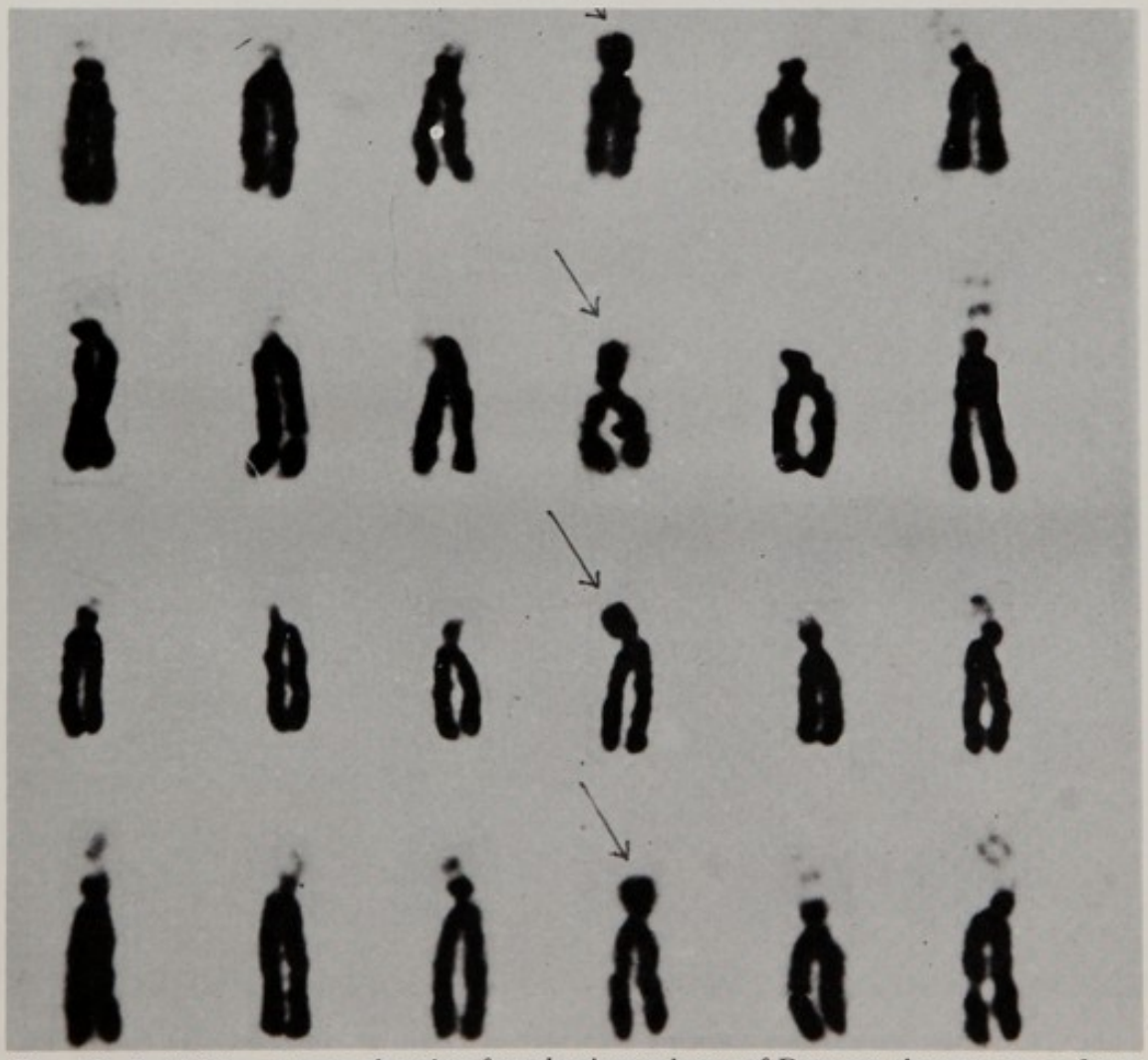


Fig. 10. Partial karyotype showing four horizontal sets of D group chromosomes from a normal man, to show: (first) Normal satellites (well shown on chromosomes 1-3 and 5 from the left in the bottom row). (second) "Double satellites" (seen on the righthand chromosome in each row); (and finally) Enlarged short arm (seen in the 4th chromosome from the left in each row, arrowed). From Engmann (1971), by courtesy of the author.

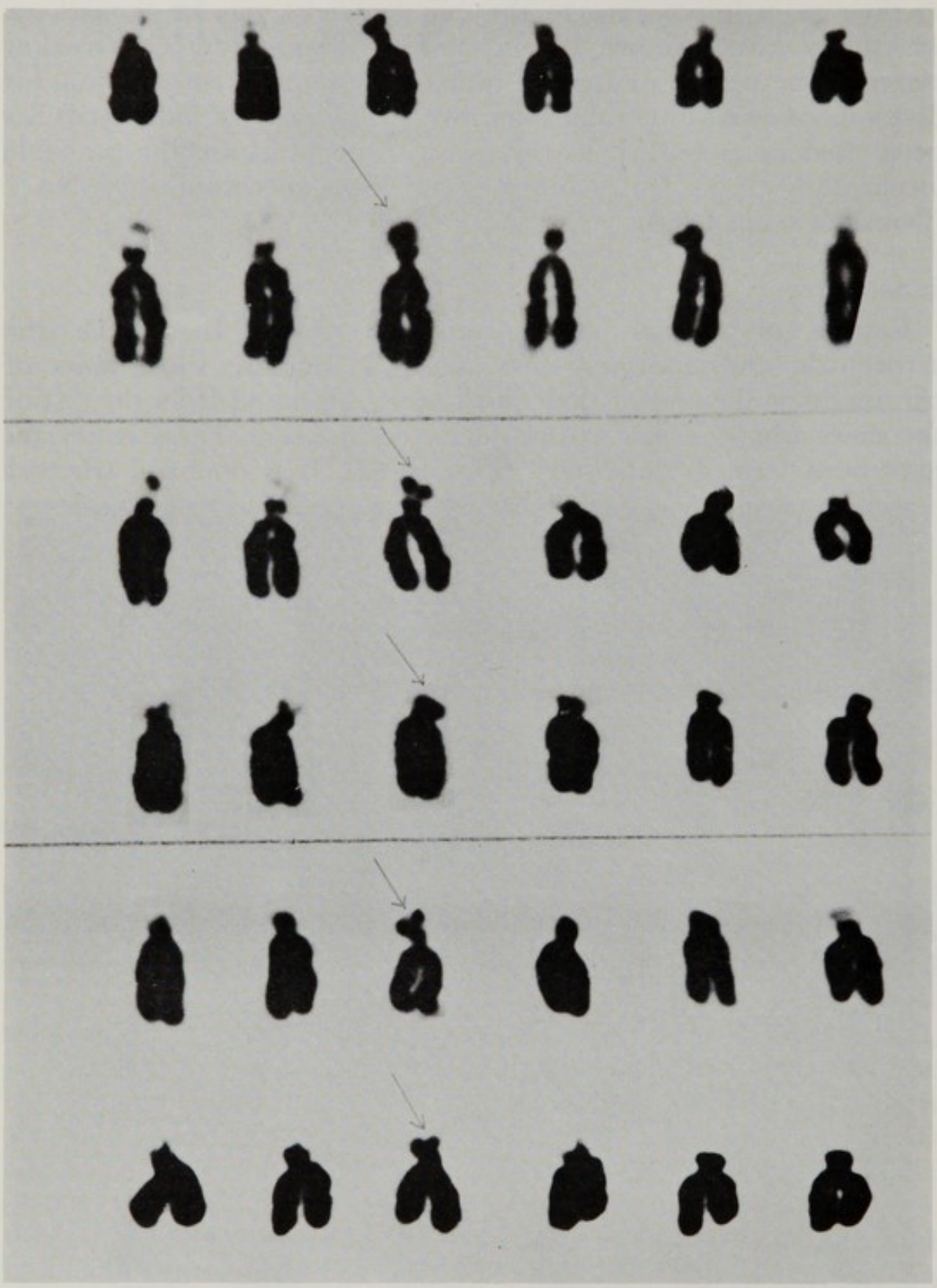


Fig. 11. Partial karyotypes showing two sets of D group chromosomes from each of three siblings. The top two sets, a and b, are from monozygotic twins; the bottom set, c, from their sister. Enlarged ("giant") satellites are present on the third chromosomes from the left in each set. Similar "giant" satellites were also present in the mother, but not the father. All individuals were phenotypically normal. From Engmann (1971), by courtesy of the author.

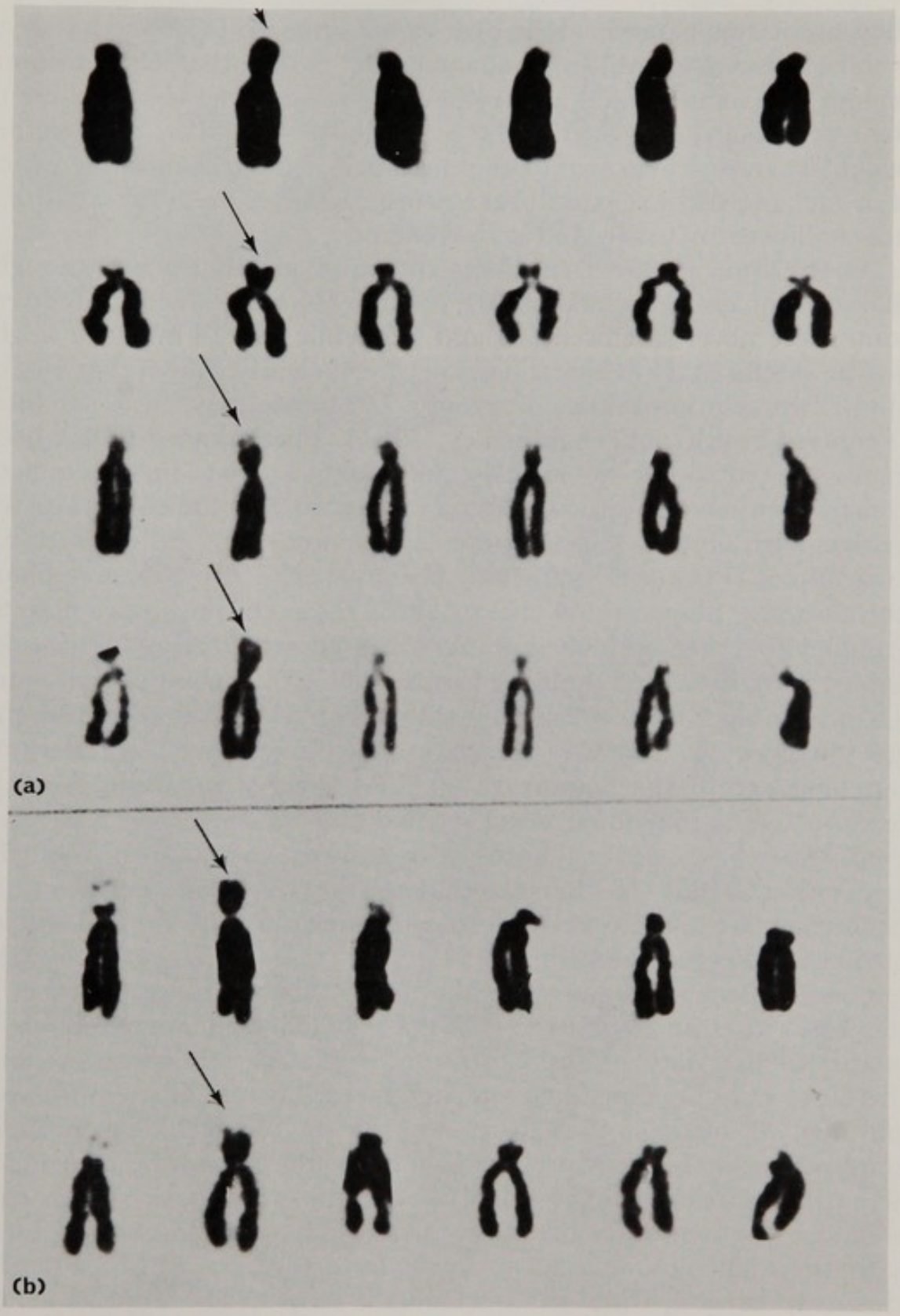


Fig. 12. Partial karyotypes showing the D group chromosomes of a mother (a, four sets) and of her son (b, two sets); in each set there are enlarged short arms on the second chromosome from the left, presumably inherited by the son from his mother. Both individuals were phenotypically normal. From Engmann (1971), by courtesy of the author.

they merit this name in their own right, since no evidence has been adduced that they differ fundamentally from other chromosomal regions except inasmuch as they have a distinctive appearance due to their terminal position. It is the pronounced secondary constriction which separates them from the remainder of the chromosome which is in fact the distinct structural feature. However the term satellite is now hallowed by usage, and is convenient.

At the time of the Denver classification in 1960 it was thought that certain chromosomal pairs (13 and 21) were always satellited, some were never satellited (15 and 22) while pair 14 might or might not be satellited. However it has since been clearly shown that any or all of the chromosomes of groups D and G may bear satellites (Ferguson-Smith and Handmaker, 1961). Furthermore it has been demonstrated both in meiosis and mitosis that the secondary constriction next to the satellite is related to the nucleolus. The ten acrocentric chromosomes show a phenomenon called *satellite association* (Ferguson-Smith and Handmaker, 1961). In metaphase chromosome preparations any or all of these chromosomes may be grouped together with their short arms and satellites in association, almost as if they were holding hands (Fig. 13). A good deal of work has been done on the frequency with which particular chromosomes are involved in satellite association, without any very definite outcome except the demonstration that there is variability both in relation to the individual being studied and the spreading technique used. There is evidence both from mitosis and meiosis that the association is due to the fact that all the secondary constrictions concerned are closely related to the nucleolus up to the end of prophase (Ferguson-Smith, 1964), and that it is probably the remnants of the nucleolus which hold these chromosomes together in this way. Satellite association is thus a secondary phenomenon which relates to the state of the cell prior to fixation and spreading, and therefore may be dependent on such factors as variability in number and size of nucleoli present as well as on the technical methods employed in making the preparation. It should perhaps be mentioned here that classical studies on plant chromosomes have shown that secondary constrictions on these chromosomes are closely associated with the nucleolus, and act as *nucleolar organizers*; in other words, after cell division, when the nucleolus is reformed, it appears at the site of these constrictions and under their influence.

It has been shown that the number of visible satellites is characteristic of the individual (Miller *et al.*, 1962; E. Ford and Woollam, 1967). In some individuals all ten acrocentric chromosomes can be seen to be satellited in some cells, and all ten

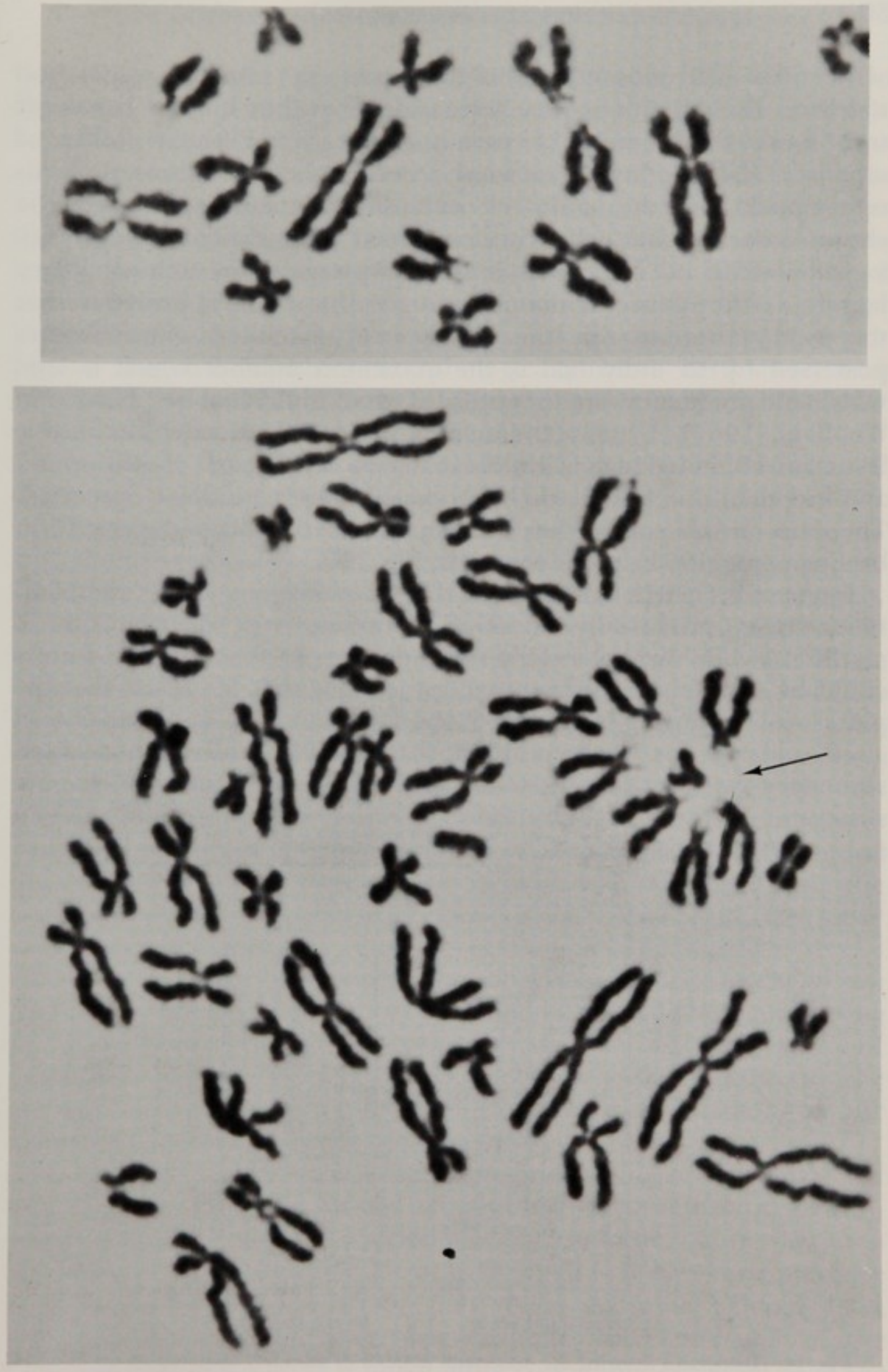


Fig. 13. "Satellite association". (top) Two D group chromosomes associated by their satellited short arms. (bottom) Association of seven acrocentric chromosomes (six Ds and one G). ×2800.

acrocentric chromosomes can take part in satellite association together, though this is very rare, and the author himself has never seen more than nine take part together. Since the visibility of satellites and of their associated secondary constrictions depends very much on the state of extension or contraction of the chromosomes in the cell being examined (and also on the physiological state of the cell, which in turn may depend on such secondary factors as the nature of the cultural conditions), it is probable that the best criterion for the number of satellited chromosomes possessed by an individual is the maximum number which is seen with any frequency in the cells of that individual (E. Ford and Woollam, 1967). Usually the maximum number of satellites seen is less than 10; individuals with less than two satellited D chromosomes and less than one satellited G chromosome have not been described. The data on this subject has been summarized in diagrammatic form by Engmann (1971) and is shown in Fig. 14.

Engmann (1967) has shown that monozygous (MZ, identical) twins have statistically identical numbers and distribution of satellites, while dizygous (DZ) twins do not, so that satellite counts could be utilized as an additional test for zygosity. It is clear that the pattern of satelliting is inherited, and Engmann (1971) has produced good evidence for the hypothesis that satellites and the associated secondary constrictions (or *stalks*, as they are sometimes called) are structural features of chromosomes which segregate in Mendelian fashion. This is supported by a number of reports in the literature

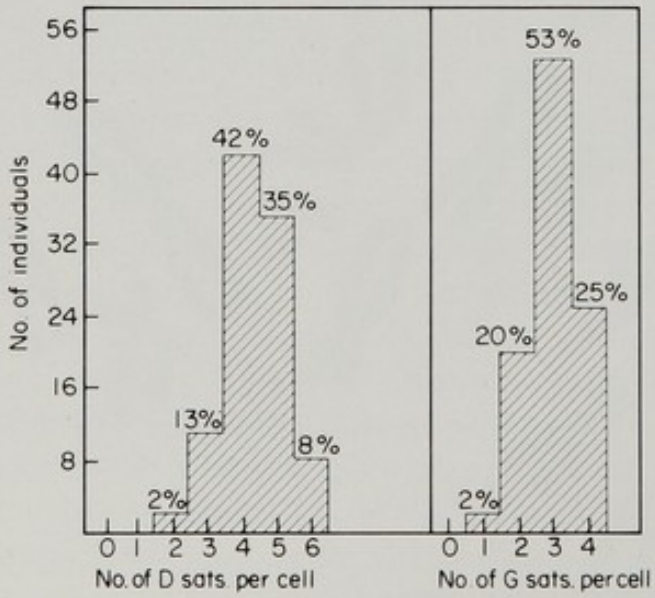


Fig. 14. Histograms showing the maximum number of satellites observed on the D and G group chromosomes in any cell in each of 100 unrelated individuals. From Engmann (1971), by courtesy of the author.

(e.g. Cooper and Hirschhorn, 1962; Ellis and Penrose, 1961; Hamerton *et al.*, 1965) of morphologically distinct satellites which have been found in two or more generations of a family, and have clearly been handed down. In several such instances other abnormalities (such as mental defect) have been found in the bearers of the distinctive satellites, but there is usually no evidence that this is anything but coincidental. In a recent study of the Old Amish, a highly inbred and isolated religious community in the U.S.A., it has been shown that some 10% of members of the community possess a "giant" satellite which is morphologically quite distinctive, and which was presumably inherited from a common ancestor of all the individuals concerned (Bahr and Golomb, 1971).

Abnormally large, or "giant" satellites (Fig. 10) are found in a proportion of normal individuals (perhaps 1-2%), although as no satisfactory definition of a giant satellite has been made in quantitative terms it is difficult to be very precise on this point; the increased size may be manifested in greater length, greater breadth, or both. Double satellites (in which the satellite itself appears to be in two parts, with a constriction between (Fig. 11)) are rarer; however Engmann has shown that double satellites are often not seen as such, but simply appear as rather large single satellites unless the chromosome concerned is very extended.

Engmann (1971) has also made extensive measurements on satellites and on the remainder of the short arms of the chromosomes which bear them, and finds that if the satellite is enlarged, the short arm remaining is correspondingly shorter, which suggests that the total amount of chromosomal material present is probably fairly constant, and that the variable feature is the position of the constriction which separates the satellite from the remainder of the short arm. However other findings, to be discussed later, concerning the possible presence in the region of satellites and their stalks of redundant genetic material, suggest that a real variation in the amount of chromosomal material present in this region of the acrocentric chromosomes is quite possible.

It will be clear from what has been said so far that the chromosomal satellites are not of great use in chromosome identification because their incidence is so variable, both between individuals and in the cells of one individual. However their presence may indicate the probability that a chromosome belongs to a particular chromosome pair. For example, since one pair of the chromosomes of group G is much more commonly satellited than the other, the presence or absence of a satellite can indicate a statistical

probability that a chromosome belongs to one or the other pair. However the interest of the human chromosomal satellites and their associated secondary constrictions lies far more in their possible use as chromosome markers for genetic linkage studies than in their use for the identification of individual chromosomes; more powerful methods of chromosome identification are now available.

Satellites are occasionally seen on other chromosomes than those of groups D and G, in normal individuals; most commonly on the short arm of chromosome No. 17. This is a rare variant, and the way in which it arises is not yet known. Where familial studies have been made on such unusual satellites, it has been shown that they are, like the satellites of group D and G chromosomes, heritable features, which can be used as markers.

3. Heterochromatin

Few terms in the cytogenetic jargon have led to greater confusion than this one. While the original concept of what heterochromatin is was a simple one, subsequent workers have tended to make their own definitions of the term, so that in reading any particular paper which refers to it, it is necessary to make quite sure what the author means by the term.

The concept was first introduced by Montgomery (1904). He observed that the X chromosomes in the germ cell line of certain insects condensed prematurely and then existed in a very contracted and densely-staining condition. He referred to these as heterochromosomes (or as showing heteropycnosis), while the normally-staining chromosomes were called euchromosomes. Subsequently, in 1928, Heitz showed that regions within individual chromosomes might exist in the same contracted state as Montgomery's heterochromosomes, while other regions in the same chromosomes did not. He called the chromatin in the two types of region *heterochromatin* and *euchromatin* respectively, and this was the original meaning of the terms.

Heterochromatin thus defined is probably derived from euchromatin by the latter passing into a permanent or semi-permanent state of condensation (Coleman, 1943), and electron microscopic studies indicate that there is no difference in the basic structure of chromatin in eu- and heterochromatic regions; any difference in appearance is due to tighter packing of the material in heterochromatic areas. It is not yet known what chemical or physicochemical factors underlie this difference in packing.

Since the original definition of heterochromatin was made many other concepts have been grafted on to it. It is now no longer possible to give any clear definition of heterochromatin, as the term is now used, other than to say that it is chromosomal chromatin that behaves differently in some way from a hypothetical typical chromatin, the euchromatin, which makes up the bulk of the total chromosomal material.

From the cytological point of view the most conspicuous feature of heterochromatin is that it is in a different state of condensation from the euchromatin. While it is usually more condensed (*positively heteropycnotic*) the term has now been extended to include chromatin that is less condensed than usual (*negatively heteropycnotic*), such as is seen in constricted areas of chromosomes.

Heterochromatin is believed to have the following metabolic properties (Brown, 1966): it is genetically inert, inactive in RNA synthesis, and it replicates late in interphase in comparison with euchromatin. Apart from these major features it has been described by various authors as having many other attributes. Since many of these relate to the postulated chemical nature of heterochromatic regions of chromosomes, it will be necessary to return to the subject later, when the structure of chromosomes has been considered in greater detail.

Heterochromatin can be considered as falling into at least two distinct categories; *facultative* heterochromatin which is the type found in the inactive member of the X chromosome pair in women and which acts as a mechanism to produce *dosage compensation* (Chapter 7); and *constitutive* heterochromatin which is present in all the chromosomes in varying amount. This latter may fall into two categories; constitutive heterochromatin which consists of redundant or repetitive DNA sequences, and which is therefore presumably fixed in position in all the chromosomes, although it may only appear at some stage of embryonic life; and constitutive heterochromatin which appears to condense during development and is perhaps related to the differentiation of tissues, so that it may vary in position and amount from one tissue to another. Yasmineh and Yunis (1970) refer to this as *condensed* chromatin rather than as constitutive heterochromatin, but the distinction is not entirely clear.

Heterochromatin is not readily seen in human metaphase chromosomes when ordinary staining methods are used; however in extended early metaphase chromosomes certain areas appear more

obviously darkly-staining than the rest, and probably represent the heterochromatin. Such areas are seen in many autosomes near the centromere and at the ends of the arms, the *"telomeres"*. In addition, most of the long arm of the Y chromosome appears heterochromatic, and at certain phases in the cell cycle one of the two X chromosomes of a woman appears heterochromatic.

During interphase, the chromatin in the cell nucleus can sometimes be seen to be condensed and dark-staining in some areas, the *chromocentres*. It has been observed, particularly in species other than man, that as the chromosomes become visible at the beginning of prophase, these chromocentres persist, and are recognizable as the heterochromatic areas of chromosomes. In women but not in men there is one especially large mass of condensed chromatin in the interphase nucleus, lying in most (but not all) tissues in contact with the nuclear membrane. This is the *sex chromatin* or *Barr body* (Barr and Bertram, 1949), which has been shown to consist of either the whole or the greater part of one of the two X chromosomes. Further discussion of the problem of heterochromatin will be found later in this chapter, and in the chapters on the meiotic chromosomes and on chromosome structure.

E. Demonstration of a Pattern Along the Length of Chromosomes

1. Introduction

Although some workers have expressed the opinion that a linear pattern can be seen on chromosomes after standard staining techniques, this has not yet been convincingly demonstrated for the human chromosomes. Although it is known that there are chemical differences in DNA in different regions of chromosomes, it is not to be expected that these would be visible to the light (or even the electron) microscope. What is known of the way in which DNA is packed into chromosomes (which suggests a complex and possibly somewhat random folding and coiling) does not suggest that linear differences in DNA structure will necessarily be reflected in linear differences in chromosome structure except in special circumstances (for example in the polytene chromosomes of certain insects (*q.v.*)) and perhaps to some extent in all meiotic prophase chromosomes. Thus any differences in staining which may be seen in different chromosomal regions will probably reflect much grosser structural differences; differences in gross coiling, and in heterochromatinization, whatever that may involve. And such structural differences

are not sufficiently constant or demonstrable by normal staining methods to be of much use in identification.

Techniques are, however, now available which can show up structural or physiological differences along the length of chromosomes, and by the use of which it may be possible to identify individually each chromosome in the human diploid set. These are (a) autoradiography and (b) specific staining techniques, particularly either with fluorescent dyes such as Quinacrine, or with the Giemsa (and possibly other) stains after various special pretreatments.

2. *Autoradiographic Analysis of Human Chromosomes*

(a) Technique. The technique of *autoradiography* depends on the ability of living tissues to take up certain substances which can be "labelled" with radioactive isotopes. These substances can then be localized on a subsequently-made slide preparation by covering the surface of the slide with a photographic film which will record the sites of radioactivity. Excellent reviews of the use of the method in cytogenetics have been written by Schmid (1963) and Miller (1970) and therefore it is only intended here to summarize what the technique has to offer in the identification of human metaphase chromosomes.

The compound which is almost invariably used in routine work is *tritiated thymidine.* Tritium is the radioactive isotope of hydrogen (^{3}H) with a half-life of 12.26 years. It has two great advantages over other isotopes of elements which may be used in biological work (such as carbon and phosphorus). It emits only α-particles which have a very short range; in water the average range is 1.5 μ with a maximum of 2 μ; and in practice, using photographic emulsion, 99% of particles have a range of less than 0.8 μ. And it is easily incorporated into thymidine, which is a precursor of thymine, one of the prime constituents of DNA. If thymidine is added to living cells which are passing through the S phase it is taken up into the new DNA which is being formed at that time.

Cells may be labelled by adding tritiated thymidine to a culture during the S period. The labelling may be either continuous (allowing the cells to remain in contact with the radioactive thymidine until the preparations are made) or *pulse* labelling; that is, exposure to the radioactive thymidine for a short, fixed period, after which the excess thymidine is removed, and the cells allowed to continue to grow in culture without it until the preparations are made. While pulse labelling has been much used, and is a valuable research technique, particularly in cultures where cell division has been synchronized, continuous labelling has been found more valuable for

chromosome recognition in asynchronized cultures. This is because the most useful part of the S-period for chromosome recognition is the last 2 hours. Before this time too many sites on most chromosomes are taking up the label to allow satisfactory discrimination; but during the last 2 hours a number of chromosomes show distinctive labelling patterns. If colcemid (*q.v.*) is added to the culture 4 hours after the beginning of labelling (corresponding approximately to the length of the G_2 period) the first cells to be arrested at metaphase will be those that had almost reached the G_2 period when labelling began. As time progresses, cells which have been labelled earlier in the S period will enter metaphase; thus by varying the length of colcemid treatment a higher proportion of early or late DNA replication stages can be obtained (Schmid, 1963).

The photographic film used is normally either a stripping film (such as Kodak AR 10, which has a 5 μ layer of film with a 10 μ layer of supporting gelatine) or an emulsion into which the slide is dipped to coat it. In either case the film used is essentially a thin layer of gelatine containing silver bromide particles, which for this purpose need to be rather fine in relation to ordinary photographic film (a particle size of 0.03-0.4 μ being usual). The film is laid over the chromosome preparation and exposed for a fairly long period in a lead-lined box which protects the film against light and background radiation. It is then developed *in situ*, when the silver grains precipitated by the impact of the α-particles can be seen in relation to the chromosomes from which the particles were emitted. Since the particles can travel at any angle from their place of origin, they will not necessarily overlie it exactly; but they will mostly lie within 1 μ of it. The *resolution* of the method (that is, the distance that must separate two labelled sources before they can be distinguished) is about 1 μ for light microscopy, and about 500 Å for electron microscopy (because the film employed is so much thinner).

Identification of individual chromosomes by their labelling patterns is accomplished by studying (i) the number of silver grains seen over them and (ii) the distribution of these grains.

Autoradiography is a laborious technique; there are several technical difficulties (such as the proneness of the films used to fog on storage due to background radiation, or through static electricity when the films are being made); the films require prolonged exposure for several days; and the counting of the silver grains is time consuming, and the interpretation of the counts sometimes difficult. For these reasons it has mainly been used as a research technique; its use as a routine has probably been mainly confined to the

recognition of the late-labelling X chromosome, which is easy even under adverse conditions because the labelling is so heavy, and is useful in the diagnosis of sex chromosome aneuploidies (Fig. 15).

Other nucleic acid precursors can be used in place of thymidine; for example ^{3}H-5-uridine is almost a specific precursor of RNA, and can be used to label it; and labelled cytosine, guanine, or 5-methyl-cytosine can be used to label DNA and RNA together, or DNA alone if RNA is previously removed with RNase; but these substances have been little used except for rather sophisticated research purposes.

(b) Chromosome recognition by autoradiography. (i) Group A. These chromosomes replicate fairly synchronously and cannot be readily distinguished by labelling pattern; nor is there a marked difference between the two arms of the metacentric members of the group, 1 and 3. Luckily these three chromosomes can normally be distinguished on morphological criteria alone. Where this is not possible (where numbers 1 and 3 are close in size) autoradiography is of no assistance.

(ii) Group B. The long arms of two of the four chromosomes of this group terminate DNA synthesis later than the other two and are thus more heavily labelled in terminally-labelled preparations. The reverse is true of the short arms. If these two are homologues (which seems likely *a priori,* although it is hard to rule out completely the hypothesis that each pair has one early and one late replicating member) then the two pairs can be distinguished from each other. The chromosome whose short arm is deleted in the cri-du-chat syndrome (*q.v.*) has consistently been found to be the one whose long arm replicates early, and careful length measurements confirm that this chromosome is No. 4 (Miller *et al.,* 1966; Warburton *et al.,* 1967).

(iii) Group C. No autosome in this group has yet been positively identified by the use of autoradiography; the only member of the group which can be identified is the late-labelling X.

(iv) Group D. The six chromosomes in this group can be divided into three pairs on the basis of their terminal labelling pattern. These have been called D1, D2 and D3 (Yunis *et al.,* 1964) or A, B and C (Gianelli and Howlett, 1966). The longest pair (A, D1) is quite late-replicating in the distal and sometimes the middle third of the long arm; the next largest (B, D2) terminates DNA synthesis only slightly earlier, but has a different labelling pattern, with late replication in the centromeric region. The third (C, D3) terminates synthesis even earlier, again with a late-replicating segment in the centromeric region. In eight cases of the D trisomy syndrome

Fig. 15. Late-labelling X chromosome. Autoradiography of a cell at metaphase after labelling with tritiated thymidine. Silver grains can be seen in relation to a number of chromosomes, but particularly to the presumed late-labelling X (arrowed). The karyotype below shows the individual chromosomes unlabelled (above) and labelled (below), for comparison. Reproduced with permission from Dr G. P. Clein.

(Patau's syndrome, *q.v.*) it could be shown each time that the trisomic chromosome was No. 13 (D1).

(v) Group E. DNA replication patterns have proved helpful in identifying the chromosomes of this group. Chromosome No. 16 has a late-replicating region in the proximal part of one arm (probably the long arm); No. 17 is the first pair in this group to terminate DNA synthesis; No. 18 is generally late-replicating, which is especially noticeable because it is a small chromosome, and these usually start synthesis late and finish early (see Chapter 6). While the chromosomes in this group can often be distinguished by morphology alone (particularly No. 16), often they cannot, and autoradiography is useful (Gianelli and Howlett, 1967).

Miller (1970) states that only one case of E-trisomy (Edwards' syndrome, *q.v.*) has been adequately studied by autoradiography. The result confirmed the general impression that the condition is due to trisomy-18, but more evidence is desirable before trisomy-17 can be completely excluded.

(vi) Group F. The intractable little chromosomes of this group are as indistinguishable by their labelling patterns as they are on morphological grounds.

(vii) Group G. These chromosomes are all very small, and appear to terminate DNA synthesis almost synchronously, but it has consistently been found that one pair is more heavily labelled than the other, which has been interpreted as due to slightly later labelling. Most workers have found that it is a member of this pair which is trisomic in Down's syndrome (mongolism); and that this chromosome pair is in fact the shorter of the two. In other words, Down's syndrome should really be described as trisomy 22. However the traditional description of the condition as trisomy 21 is felt to be so deeply imprinted on the minds of cytogeneticists that it would be confusing to alter it at this late stage. The replication pattern of the Philadelphia (Ph_1) chromosome (*q.v.*) has been studied in six cases. In three it was late-replicating, in three indeterminate. The chromosome is so small that it is perhaps difficult to compare it to a normal chromosome of this group by autoradiography. In any case, fluorescent studies seem to indicate that it is a member of the other pair than that involved in Down's syndrome (see later).

(viii) Y chromosome. This has been found to be usually, but not always, later-replicating than chromosomes 21 and 22.

(ix) X chromosome. One chromosome of the C group is found, in normal females, to synthesize DNA very late; to be still active, in fact, after most of the other chromosomes have finished replicating.

No such late-replicating chromosome is seen in normal males, or in females with Turner's syndrome, who have only one X chromosome (45, X). Conversely, individuals believed to have additional X chromosomes (47, XXX, 48, XXXY, etc.) have additional late-labelling C-group chromosomes, one less in number than their presumed complement of X chromosomes. There seems to be no doubt that these additional late-labelling chromosomes are X chromosomes, and that they correspond to the heterochromatic X chromosomes which form the sex chromatin bodies (Chapter 7).

(x) Summary. Autoradiography has proved especially useful in the identification of chromosomes 4 and 5, chromosomes of the D, E and G groups, and the late-labelling X chromosome. It can also be used to help identify the Y chromosome. It is of little help with chromosomes of groups A, C and F. It is probably too laborious a procedure for use in the routine identification of chromosomes, except for the late-labelling X, and is likely to be superseded for this purpose by the staining methods about to be described. It can be useful in the identification of translocation chromosomes, particularly those involving the D and G groups, but here again the newer staining methods promise to be generally more convenient.

3. *Fluorescent Staining in the Identification of Metaphase Chromosomes*

It has been known for some time that certain acridine derivatives such as acriflavine or acridine orange react with nucleic acids to produce a bright fluorescence when viewed with an ultraviolet light source. It has recently been shown by Caspersson *et al.* (1969a) that certain acridine derivatives, notably quinacrine mustard (QM) and quinacrine hydrochloride (Q) can produce specific banding patterns on both plant and animal chromosomes, which can be used in their identification (the work on human chromosomes is summarized by Caspersson *et al.*, 1971).

The fluorescent staining seen after exposure to aqueous solutions of QM or Q is of two different degrees of intensity. There is a very intense fluorescence of about 1-2% of the total length of the chromosome complement. This is shown by the whole of the long arm of the Y chromosome (Zech, 1969) and sometimes by the short arms and/or satellites of the acrocentric chromosome pairs 13-15 and 21-22 and the centromere region of No. 3. This intense fluorescence of the acrocentric chromosomes seems to vary both between individual chromosomes and individual people, and is probably inherited as a polymorphic feature of these chromosomes; the

intense fluorescence of the Y chromosome seems by contrast to be a constant feature, unless the Y itself is abnormally small. Borgaonkar and Hollander (1971) have shown that fluorescence may be absent in a small (but functionally normal) Y chromosome.

This intense fluorescence persists during interphase, and Pearson *et al.* (1970) have demonstrated that a distinctive fluorescent Y-body can be demonstrated in interphase nuclei of normal men. This technique offers a valuable supplement to the test for presence of a sex chromatin body in the diagnosis of aneuploidy of the sex chromosomes. It is also possible to demonstrate the presence or absence of a Y-body in spermatozoa (Sumner *et al.*, 1971a).

In addition to the intense fluorescence shown by certain chromosomes, it is found that all the chromosomes show a less intense fluorescence which varies in different regions of individual chromosomes and thus produces banding patterns along their length. These patterns differ in every chromosome pair in man, and thus provide a means by which each pair can, under favourable circumstances, be identified. Caspersson *et al.* (1970, 1971) have published graphs showing the fluorescence profile of each individual chromosome pair (Fig. 16). These profiles were made by the use of a reflectance photometer, and Caspersson and his coworkers consider that this is the method of choice for the identification of chromosomes by their fluorescence patterns, since the value of purely visual analysis is limited by the difficulty of memorizing complex banding patterns, and by the difficulty of observing subtle differences in the level of fluorescence. However other workers (O'Riordan *et al.*, 1971) claim that every chromosome pair can be identified by visual inspection alone. Visual inspection is certainly adequate to distinguish most if not all of the chromosomes involved in D/G translocations (O'Riordan *et al.*, 1971 (Fig. 17); Breg *et al.*, 1971).

Quinacrine mustard and quinacrine produce fluorescence at more or less the same chromosomal sites, but QM gives a more intense and more stable effect (Caspersson *et al.*, 1969b) and is to be preferred as a stain; however Q has been extensively used because of its readier availability and seems to give quite satisfactory results, particularly for the intensely fluorescent regions. Other fluorochromes can also be used and some give different staining patterns. Ethidium bromide, for example, seems to produce almost opposite fluorescence patterns to quinacrine; areas which are most fluorescent with this dye are those which are least fluorescent with quinacrine (Caspersson *et al.*, 1969b).

It is not yet known what causes these dyes to bind to particular

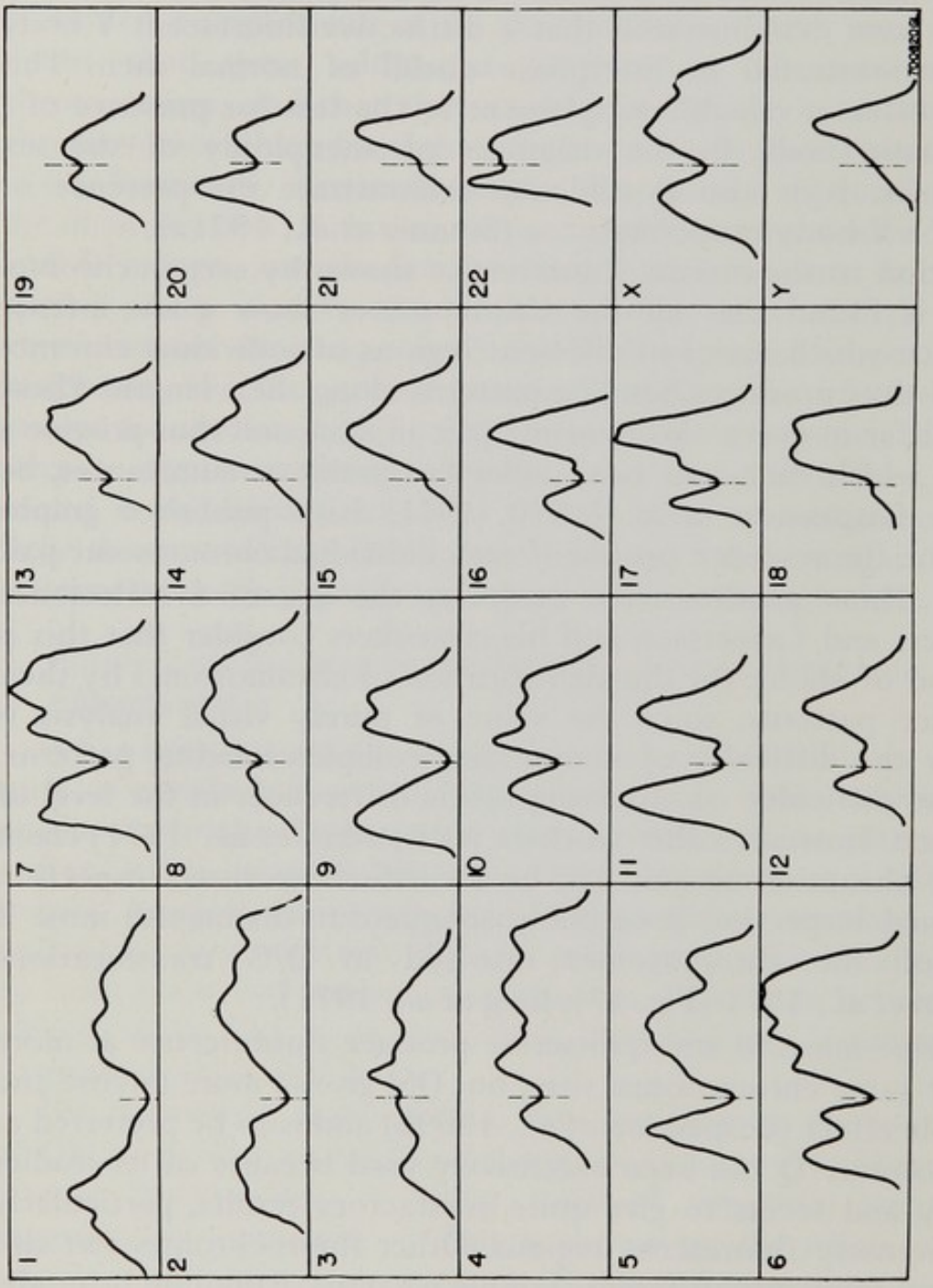


Fig. 16. Fluorescence profiles of the human chromosomes after staining with quinacrine mustard. The vertical line indicates the position of the centromere. Reproduced from Caspersson *et al.* (1970), *Expl Cell Res.* **62**, 490, by courtesy of the authors.

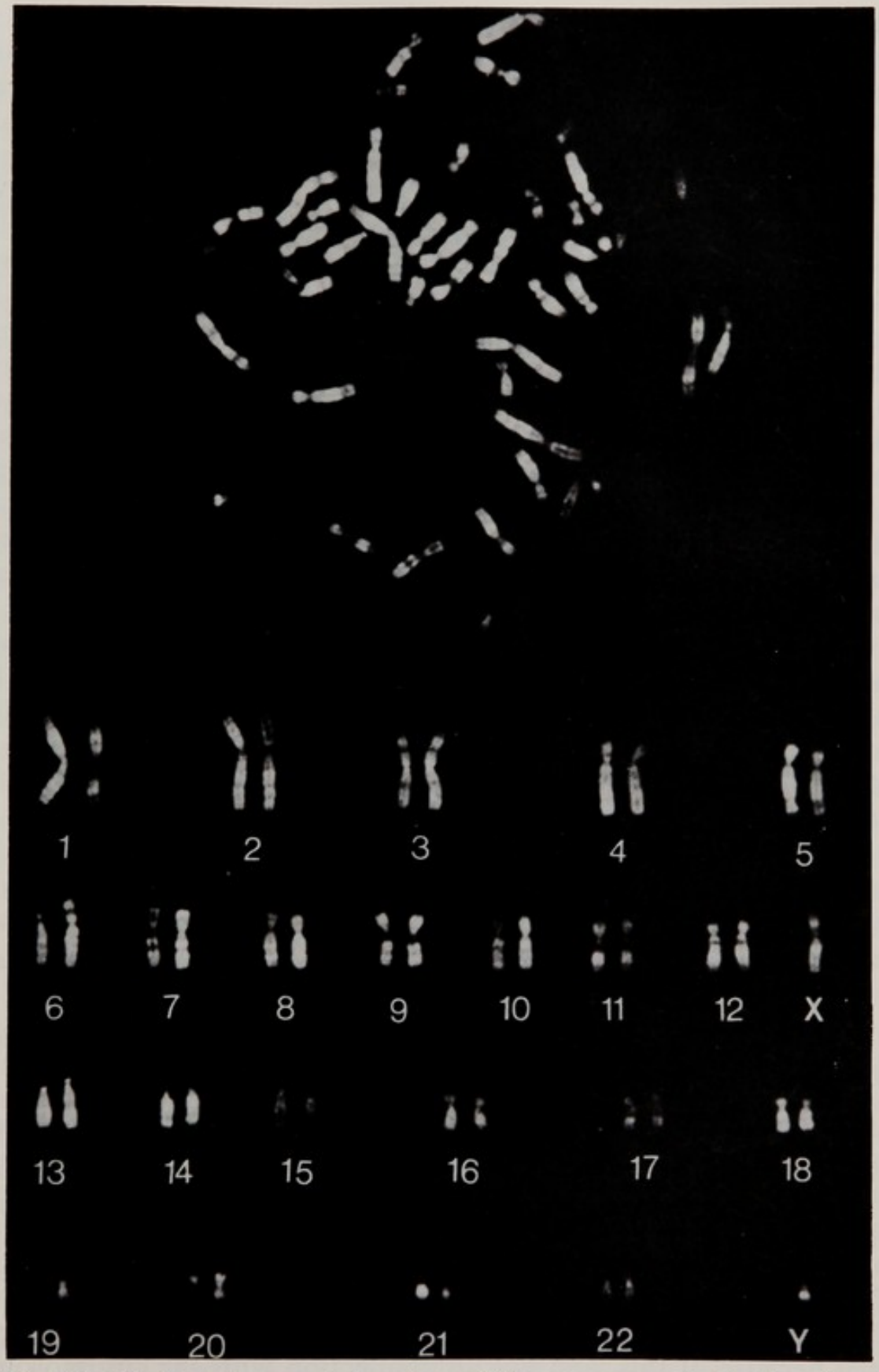


Fig. 17. Metaphase figure and karyotype of chromosomes stained with quinacrine dihydrochloride, as described by O'Riordan *et al.* (1971). Each chromosome shows a distinctive banding pattern by which it may be identified. Reproduced by courtesy of the staff of the MRC Clinical and Population Cytogenetics Unit, Western General Hospital, Edinburgh.

regions of chromosomes, though various interactions are known to be possible. Quinacrine may act by intercalation of the acridine nucleus into the grooves on the outside of the DNA double helix, and also by binding of its diaminoalkane sidechain to the phosphate groups of the DNA. Quinacrine mustard can also bind in both these ways and also by alkylation of DNA bases, particularly at the N7 atom of guanine; thus it is thought that it may be guanine-specific and bind particularly to regions of DNA with a high GC/AT ratio (Caspersson *et al.*, 1969a). However quinacrine, which does not in theory do this, seems to bind to the same sites. So it is not at all clear which of the binding methods mentioned above is most important, or whether other factors may also be involved. Nor is it yet known what produces the intense fluorescence shown by the Y and certain other chromosomes. However it is possible that some base specificity is involved, and that the guanine content of the DNA may be one important factor. The method offers the possibility of differential staining of chromosomes, and perhaps of estimating the relative base content of different regions.

There are a number of fluorochromes which are known to possess base specificity, but which do not fluoresce strongly enough to be used. For example, actinomycin and chloroquin are guanine-specific, and nogalomcyin binds to A-T base pairs; if the fluorescence of these substances could be enhanced they could provide much useful information (Caspersson *et al.*, 1969b).

The banding patterns produced after quinacrine staining will be discussed in more detail later, in relation to the banding patterns produced by Giemsa staining after special pretreatment, which will be described in the next section.

4. Giemsa Staining After Special Pretreatment in the Identification of Metaphase Chromosomes

Another potent method of differentiating between chromosomal regions has been developed as a consequence of experiments carried out on the hybridization of DNA by Jones (1970) and Pardue and Gall (1970). These workers tried to localize the positions on chromosomes occupied by a particular variety of DNA, "satellite DNA". Satellite DNA forms a distinct fraction of the total DNA which can be separated by differential centrifugation (the name is given because of this, and has no connection with the chromosomal satellites). It has a different base composition from the main bulk of the DNA (as shown by a different melting temperature—see Chapter 5) and it consists of highly repetitive base sequences (shown

by the fact that when it is denatured it renatures or "anneals" quicker than the rest of the DNA). Human DNA contains about 2.5% of satellite DNA, containing such highly repetitive base sequences, and this itself can be separated into two distinct fractions. In addition human DNA contains a further 15% of "homogeneous main band DNA" which is moderately repetitive (Corneo *et al.*, 1970).

Jones (1970) and Pardue and Gall (1970), working on the mouse, which has 10% of satellite DNA in its genome, denatured the DNA in fixed chromosomal preparations by treatment with NaOH, and then incubated in warm saline in the presence of labelled single-stranded satellite DNA or its complementary RNA in order to produce renaturing (annealing). They were able to locate the labelled satellite DNA in the centromeric regions of the mouse chromosomes, thus indicating that the mouse satellite DNA was located in these regions. Pardue and Gall also found, however, that these regions stained deeply with Giemsa stain after this procedure. They attributed this to the likelihood that the repetitious DNA in these regions had annealed more rapidly than the bulk of the DNA. If this was so, chromosomal regions which stained deeply with Giemsa after such a denaturation-renaturation procedure would be those regions that contained highly repetitious base sequences, and the technique could be used to locate such sequences within chromosomes.

Arrighi and Hsu (1971) have used this technique on human chromosomes, and have shown that deep Giemsa staining is found in the centromeric region of most of the chromosomes (as in the mouse), and also in the long arm of the Y chromosome. This seems to indicate that these regions are composed of constitutive heterochromatin containing highly repetitious base sequences.

Sumner *et al.* (1971b) have confirmed these findings, and made a further discovery. If chromosome preparations are *not* denatured after fixation, but are immediately incubated for an hour in the renaturing solution (SSC, a mixture of sodium chloride and trisodium citrate), and then stained with Giemsa stain, a banding pattern is produced which closely resembles that seen in the chromosomes in quinacrine fluorescence, and which can similarly be used in the identification of individual chromosomes (Fig. 18). They have called this the ASG (acetic/saline/Giemsa) technique.

A number of other workers have independently made and published rather similar findings (Drets and Shaw, 1971; Patil *et al.*, 1971; Ridler, 1971; Schnedl, 1971a and b; Seabright, 1971, 1972; Wang and Fedoroff, 1972). The methods used have varied both in the time of incubation and in the pretreatment procedures used, but have all resulted in banding patterns with the Giemsa stain.

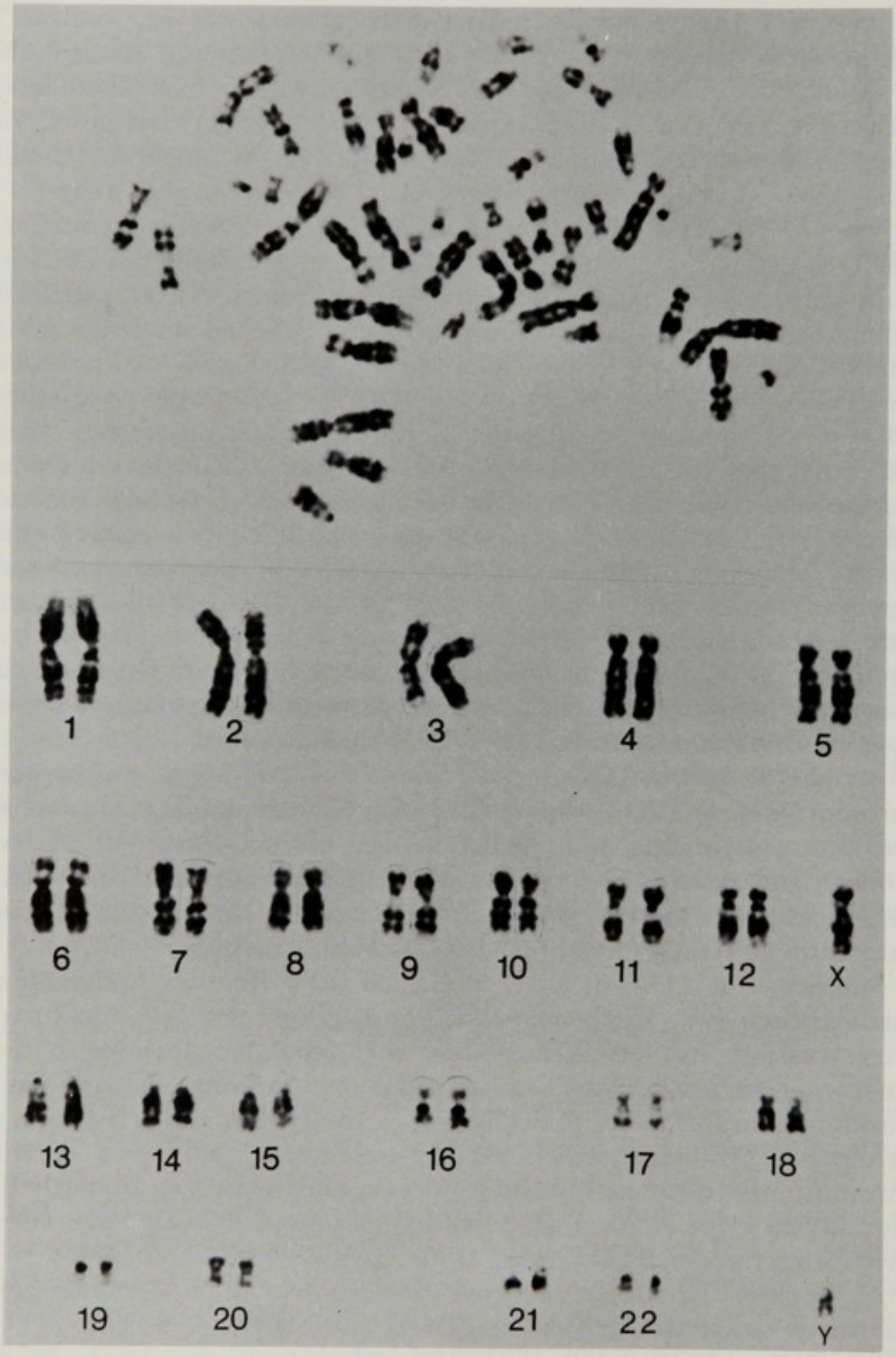


Fig. 18. Metaphase figure and karyotype of chromosomes stained by the ASG (acetic-saline-Giemsa) technique. Each chromosome shows a distinctive banding pattern by which it may be identified. From Sumner *et al.* (1971), *Nature New Biology* 232, 31, by courtesy of the authors.

The precise reason for these findings is not yet clear, but will be discussed in the next section. However, whatever the reason for the production of these banding patterns, there can be no doubt of their potential value in chromosome identification. Like the fluorescent bands seen after quinacrine staining, they can be used to identify individual chromosomes, and in particular, the chromosomes involved in translocations. While it seems likely that some polymorphism of these banding patterns may be found in different individuals, this will probably not interfere seriously with the correct identification of most chromosome pairs.

The quinacrine fluorescence techniques are simpler and quicker than autoradiography, although they involve the possession of apparatus for fluorescence microscopy, and suffer from the disadvantage that the fluorescence fades fairly fast, so that permanent preparations cannot be made. The Giemsa techniques have further advantages, notably that they demand no special apparatus, and allow the making of permanent preparations; however they are not yet quite as consistent in their end-product as the fluorescent techniques; different pretreatments may give different banding patterns; and until these techniques have become more standardized, it seems likely that fluorescent techniques will remain the method of choice for the precise identification of chromosomes and parts of chromosomes.

5. *Comparison of the Autoradiographic, Fluorescent and Pretreatment-Giemsa Methods of Chromosome Identification*

The pretreatment-Giemsa staining methods are of two main sorts; the techniques which supposedly denature and then renature the DNA result in staining of only the centromeric regions of most chromosomes, and the long arm of the Y chromosome. The other treatments, which mostly involve incubation in various strengths of SSC (sodium chloride and trisodium citrate) for various periods from one hour up to three days, sometimes in conjunction with other treatments (heat, alkali or trypsin) give a much more widespread banding pattern similar to that seen after quinacrine staining.

The experiments of De la Chapelle *et al.* (1971), using acridine orange, which fluoresces green with double-stranded DNA and red with single-stranded DNA or RNA, seem to confirm that chromosomal DNA is normally double-stranded, and that the supposed denaturation-renaturation procedures such as that of Arrighi and Hsu (1971) do indeed break down double-stranded DNA into single-stranded, and then reanneal it; this provides additional evidence for the view that the chromosomal regions which stain positively with

Giemsa after these procedures consist of repetitious satellite DNA. Freeman *et al.* (1971) have also shown that the chromatin of interphase nuclei consists mainly of double-stranded DNA except during the S period.

Several groups have now compared the banding patterns obtained on quinacrine fluorescence with those obtained by the pretreatment-Giemsa techniques (Gagné *et al.*, 1971; Evans *et al.*, 1971; Aula and Saksela, 1972). They have found that there is a very close correlation between the bands produced by the two techniques, with certain exceptions. Centromeric heterochromatin seems to stain positively with Giemsa but negatively with fluorescent techniques (chromosomes 3 and 6 are exceptions to this rule); and the secondary constrictions of chromosomes 1, 9 and 16 are always negative with quinacrine but positive with Giemsa, after alkali pretreatment. Gagné *et al.* (1971) suggest that the regions which are Giemsa-positive after denaturation and renaturation contain all the human satellite DNA sequences; whereas QM only binds to certain of these sequences, perhaps because of base specificity, thus differentiating between two distinct varieties of constitutive heterochromatin. However there are other possible explanations.

It seems in general that bands fluorescent with Q or QM (except for the intensely fluorescent regions) correspond rather closely with the Giemsa bands obtained after pretreatment with SSC, as in the ASG technique, and also with areas of chromosomes which are autoradiographically late-labelling. All three methods may be considered to show up heterochromatin of the constitutive variety. Whether the difference between this heterochromatin and the euchromatin is chemical (e.g. in DNA base composition) or physical (in the nature and density of coiling) or both is not yet clear. However since many of the late-labelling areas correspond to sites of secondary constrictions (Schmid, 1963) it seems likely that differences in coiling are amongst the factors involved.

In addition to this variety of constitutive heterochromatin there is clearly another variety present in the chromosomal regions which show intense fluorescent staining and which continue to stain with either the fluorochromes or Giemsa after denaturation and renaturation. This heterochromatin is not always autoradiographically late-labelling, and the specific staining seems likely to be due to the presence of highly repetitive DNA base sequences, which in turn is probably associated with a different AT/GC ratio from the rest of the chromatin. The DNA in these regions probably consists wholly or partly of the satellite DNA, which is thought to make up some 2.5% of the total.

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V. VARIATIONS IN KARYOTYPE IN PHENOTYPICALLY NORMAL PERSONS

A number of variations in the human karyotype have been reported in persons who are phenotypically normal. These fall into one of three categories:

(a) Variations in chromosome number (aneuploidy).

(b) Variations in the structure of individual chromosomes which do not appear to have any genetic effects, and therefore do not probably result from structural rearrangements.

(c) Variations in the structure of individual chromosomes which are due to structural rearrangements of their contents and which may therefore lead to abnormal meiosis and possibly to the production of abnormal gametes.

A. Aneuploidy in Normal Persons

It has been shown (Jacobs *et al.*, 1961, 1963) that the number of aneuploid cells found in blood cultures from normal people increases with age. The change normally consists of loss of a single chromosome. In women the process starts in the sixth decade, and eventually up to 7% of cells may have only 45 chromosomes. The missing chromosome is one of the C group, and since experience shows that cells which are monosomic for an autosome do not usually survive it seems almost certain that the missing chromosome is an X, and probably the inactive member of the pair. In men there is a comparable phenomenon, but it is less striking. There is an increase of aneuploid cells which lack a single small acrocentric chromosome, almost certainly the Y. The effect does not appear until the seventh decade, some ten years later than in women, and the number of aneuploid cells is not usually above 1-2%.

There were one or two reports, soon after karyotyping became possible, of differences of chromosome number in different races, but these failed to receive confirmation and may be discounted.

B. Variations in Chromosome Structure Without Genetic Effects

1. Variation in the Y Chromosome

Y chromosomes which are unusually long (longer than the chromosomes of the F group, and sometimes as long as those of group D), unusually short (less than half the length of the

chromosomes of the G group) or metacentric, have all been described in individuals and families who are perfectly normal, and fertile. Since the Y chromosome appears to contain so little active genetic material, it is difficult to exclude the possibility that these anomalous Y chromosomes contain structural rearrangements; but if such rearrangements are present they are unlikely to result in genetic ill effects since either the Y chromosome does not take part in crossing-over at meiosis, or if it does, only a very small segment is involved. This variation in length seems only to involve the long arm.

Unnérus *et al.* (1967) have shown that the length of the Y chromosome has a Gaussian distribution in the Finnish population, and that it is inherited at constant length (in relation to the length of the E group chromosomes). The length distribution may be different in different races. Cohen *et al.* (1966) have shown that long Y chromosomes are more common in the Japanese than in other racial groups studied. In Britain a Y chromosome longer than the F group chromosomes is found in about 2% of men (Court Brown, 1967); a short Y is rarer, but there are not enough data to estimate the incidence accurately. Another variant which occurs, but is rarer still, is a small metacentric Y resembling that found in some great apes. Since these unusually-sized Y chromosomes are passed on from father to son they may be used as additional evidence in the rare cases of disputed paternity where the putative fathers have Y chromosomes which differ markedly in length.

2. Variation in the Autosomes

This occurs mostly in autosomes which have prominent secondary constrictions. The commonest variations are in the short arms and the satellites of the acrocentric chromosomes of groups D and G. Variation in the appearance of the satellites and their stalks have already been described. Apart from this, a short arm of one of these chromosomes which is unusually large (with or without a satellite) is seen in between 1-3% of persons (Fig. 11). Since it is possible to have variation in short arm length, in satellite size, and in size of secondary constrictions in these arms, without any effect on the phenotype of the individuals concerned, it seems likely that these regions may be sites of redundant DNA. This is borne out by the way in which they stain with fluorescent dyes like quinacrine and with the denaturation-renaturation Giemsa technique. The DNA may be satellite DNA, and the constrictions (and possibly the rest of the short arms) may contain DNA coding for ribosomal RNA (as shown

by the function as nucleolus-organizer). It may be that the number of repetitious sequences which do this vary in individuals, and that the total number is not very critical.

Chromosome 16 has often been seen to be rather variable in size, and one member of the pair is sometimes conspicuously larger than the other (sometimes as large as a C group chromosome). This was found by Court Brown *et al.* (1966) in 2/438 normal individuals.

Another variant which has been regularly if rarely seen is a satellited chromosome 17 or 18. This was found by Court Brown *et al.* (1966) in 3/756 persons. In one of these the anomaly appeared to have arisen as a consequence of a reciprocal translocation between the short arm of a G group chromosome and the 17 or 18 chromosome, and in two members of the same family an unbalanced translocation of the same type was found without apparent phenotypic abnormality. This suggests that some if not all satellited chromosomes 17 or 18 have appeared as a consequence of such a translocation.

Other variants which have been described are alterations in the arm ratios of chromosomes such as Nos. 1 or 9, which contain prominent secondary constrictions; these alterations are due to lengthening of the arm containing the constriction. It seems that genetically determined alterations in coiling can exist at the sites of secondary constrictions, and can give rise to these differences in length. These alterations can be inherited. The elongation sometimes seen on chromosome No. 1 is of particular interest because it enabled the first localization of a genetic locus on a particular autosome to be made (Chapter 14), when linkage was demonstrated between the uncoiling and the locus for the Duffy blood group (Donohue *et al.*, 1968).

In short, most of the variation in autosomal morphology is due to variation at sites where secondary constrictions are found. This may be due to the fact that these are sites of repeating or redundant DNA, which either has unusual coiling properties, or may contain deletions or duplications which produce no obvious phenotypic effect because of the nature of the DNA involved. The more carefully the human metaphase karyotype is examined, the more evident it seems that most of these variations are heritable polymorphisms of the chromosomes, which may be expected to vary in incidence both in individuals and in different ethnic groups, so that many individuals can be differentiated from each other by means of these structural variations in their chromosomes (Leisti, 1971).

C. Variations in the Karyotype of Phenotypically Normal People Due to Structural Rearrangements of Chromosomes

Court Brown (1967) estimated that 0.5% of phenotypically normal adults showed chromosomal structural heterozygosity (that is, possessed a structurally abnormal autosome) which could be detected. In a later study the figure was revised to 0.3% (14/4833 adults), but the latter group contained a number of young adult males from institutions and was not therefore completely randomly ascertained. However the figure represents the best estimate available. Of 11 abnormal autosomes found, six were translocations between D group chromosomes of centric fusion type, suggesting that this is the commonest autosomal structural anomaly present in the population at large. The incidence of this karyotype, 45,D-, t(DqDq)+ is about 1/800. Nearly all the other autosomal heterozygosities were due to translocations; only one autosomal inversion was seen.

The incidence of detectable inversions (and only pericentric inversions which alter the arm ratio can be seen in mitotic preparations) appears to be very low. Waltzer *et al.* (1969) found three in 2400 infants, and two of these involved the Y chromosome. But for the reason just given, the actual incidence of all types of inversion must be appreciably higher than can be detected; the new differential staining techniques may reveal some inversions which could not previously be found.

In a group of newborn children three structural chromosome alterations were found in 1788 children, and two of these involved autosomes. There were ten children in the group with some form of chromosomal abnormality (Court Brown, 1967), and the remaining seven comprised three sex chromosome aneuploidies and four autosomal aneuploidies. Court Brown suggested that about 1% of newborn children might be expected to have a demonstrable chromosome abnormality; half of them structural chromosomal changes and the other half aneuploidies, equally divided between the sex chromosomes and the autosomes. This figure will no doubt be refined when larger series are available for study, but gives a good idea of the frequency of chromosomal anomalies at birth. (See Chapter 9.)

§ 3 §

Meiosis and Meiotic Chromosomes

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I. INTRODUCTION

It is a remarkable fact that hardly any good meiotic preparations were made from the human testis before 1956, when C. E. Ford and Hamerton first successfully used the Feulgen-squash technique which had been developed by Darlington and others in the late 1930s for use on plant chromosomes, and were able for the first time to demonstrate meiotic chromosomes which were so well separated from each other that they could be counted and examined morphologically. Perhaps this tardiness was largely due to the difficulty in obtaining human testicular material for purely scientific purposes, and partly also to the illusion that human meiotic preparations were particularly hard to make. Even since 1956 the amount of work on human meiosis has been scanty in comparison to that on mitotic chromosomes. Yet given the material, the making of preparations of meiotic chromosomes is no harder in man than in any other animal, and the techniques involved are quite straightforward.

II. THE CYTOLOGY OF MEIOSIS

There are considerable differences in the meiotic cell divisions between men and women; mainly in the timing and the length of the process, but also to a lesser extent in the morphology of the cells concerned. The sexes will therefore be dealt with separately.

A. Meiosis in Man

1. Development of the Testis

In both sexes the *germ cells* (that is, the cells which will give rise to the gametes) appear in the gonad (ovary or testis) in the early embryo, having migrated there from the tissue around the yolk sac. These cells have a distinctive morphology even in the early embryo, and thus can be recognized during their migration. In the testis they migrate from the outer layer (cortex) into the interior, where they, together with other cells of the embryonic mesoderm, form the *seminiferous tubules.* In an immature man or mammal the cells of these tubules are of two sorts; supporting *Sertoli cells* which mainly lie around the periphery of the tubule, and the germ cells themselves, which can be recognized by their characteristically pale cytoplasm, are fewer in number than the Sertoli cells, and mostly lie more centrally in the tubule. The Sertoli cells, which are formed from the embryonic mesoderm, later take on the function of supporting and nourishing the cells which develop from the primitive germ cells.

At the time of puberty, and under the influence of the gonadotrophic hormones of the pituitary gland, the process of meiosis is initiated.

2. The Meiotic Cell Divisions

At puberty the germ cells begin to divide and multiply continuously, and are called *spermatogonia.* At any given time certain of the spermatogonia cease to divide, and instead enlarge and enter prophase of the first meiotic division. These are the *primary spermatocytes.* During first meiotic prophase homologous pairs of chromosomes come together *(synapsis)* and crossing-over takes place between them. At the end of prophase the cells proceed to metaphase, and the *reduction division* takes place. The two daughter cells are the *secondary spermatocytes,* each of which contains only a haploid set of chromosomes; either 22,X or 22,Y. Each chromosome of these haploid sets still consists of two equal chromatids. These chromosomes do not disappear during interphase, but proceed almost immediately to second meiotic metaphase. After this, the two chromatids of each chromosome separate and pass to opposite poles of the spindle to form two daughter cells, the *spermatids.* A spermatid therefore contains only a single set of genetic material. After this the spermatids develop into *spermatozoa* by passing through a complicated series of cytological changes referred to as *spermiogenesis.*

3. The Cytogenetics of Male Meiosis

(a) Spermatogonial metaphase. The mitotic chromosomes of spermatogonial metaphases are not quite the same in appearance as those found in mitotic preparations from somatic cells (Fig. 19). The chromosomes can be identified in the usual way by size and centromere position, and can be karyotyped. But they appear rather indistinct and fuzzy, and often show coarse helical coils. The centromere may appear double. Another feature sometimes seen is polyploidy, which may appear in up to 25% of cells (McIlree *et al.*, 1966) with a mean of 7-8% (Sasaki and Makino, 1965; Kjessler, 1966). One possible explanation of this is that the polyploidy is not in fact of spermatogonia but of Sertoli cells, which are often polyploid (J. D. Boyd, personal communication). This however would hardly account for 25% of mitoses seen. Another likely possibility is incomplete separation of the spermatogonia when they divide. It has been shown (see p. 69) that spermatocytes and spermatids are normally joined to each other by cytoplasmic bridges, and it is possible that this could be true of some spermatogonia.

(b) First meiotic prophase. This is the longest and most complicated stage of the meiotic process, and is conventionally divided into five phases.

(i) Leptotene (leptonema) (Fig. 20a). At the beginning of this stage the chromosomes become visible in the nucleus of the spermatocyte as fine threads, and as the stage advances they become shorter, thicker and easier to see. Electron microscopic studies show that they are attached to the nuclear membrane at both their ends (Comings and Okada, 1970). Since they are relatively long and convoluted in relation to the size of the nucleus, and the nuclear membrane is still present, it is not possible effectively to separate them from each other at this stage.

(ii) Zygotene (zygonema) (Fig. 20b). At the beginning of this stage the single chromosome threads begin to pair with their homologues, rather like the two sides of a zip fastener coming together. The process often begins at the ends of the chromosomes, where they are attached to the nuclear membrane [Comings and Okada (1970) have shown that at the end of leptotene the ends of homologues are attached to the nuclear membrane in close proximity to each other but may begin at other points along the lengths of the chromosomes]. This process of synapsis progresses until it is complete, and the paired structures thus formed, each consisting of two homologous chromosomes, are called *bivalents.* The sex chromosomes, which are not homologous like the autosomes (except

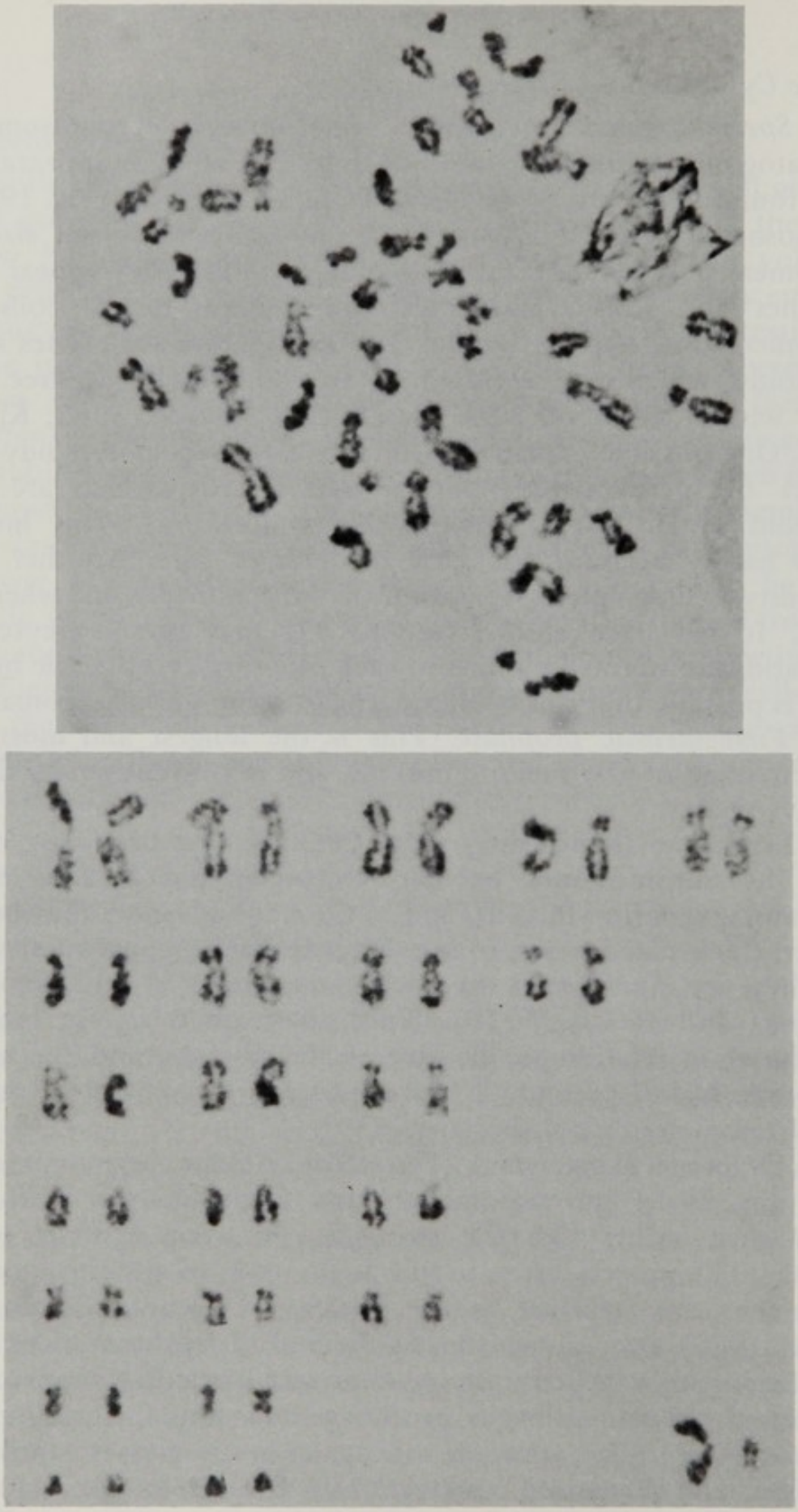


Fig. 19. Spermatogonial metaphase chromosomes, with karyotype. Reproduced by courtesy of Dr J.-M. Luciani.

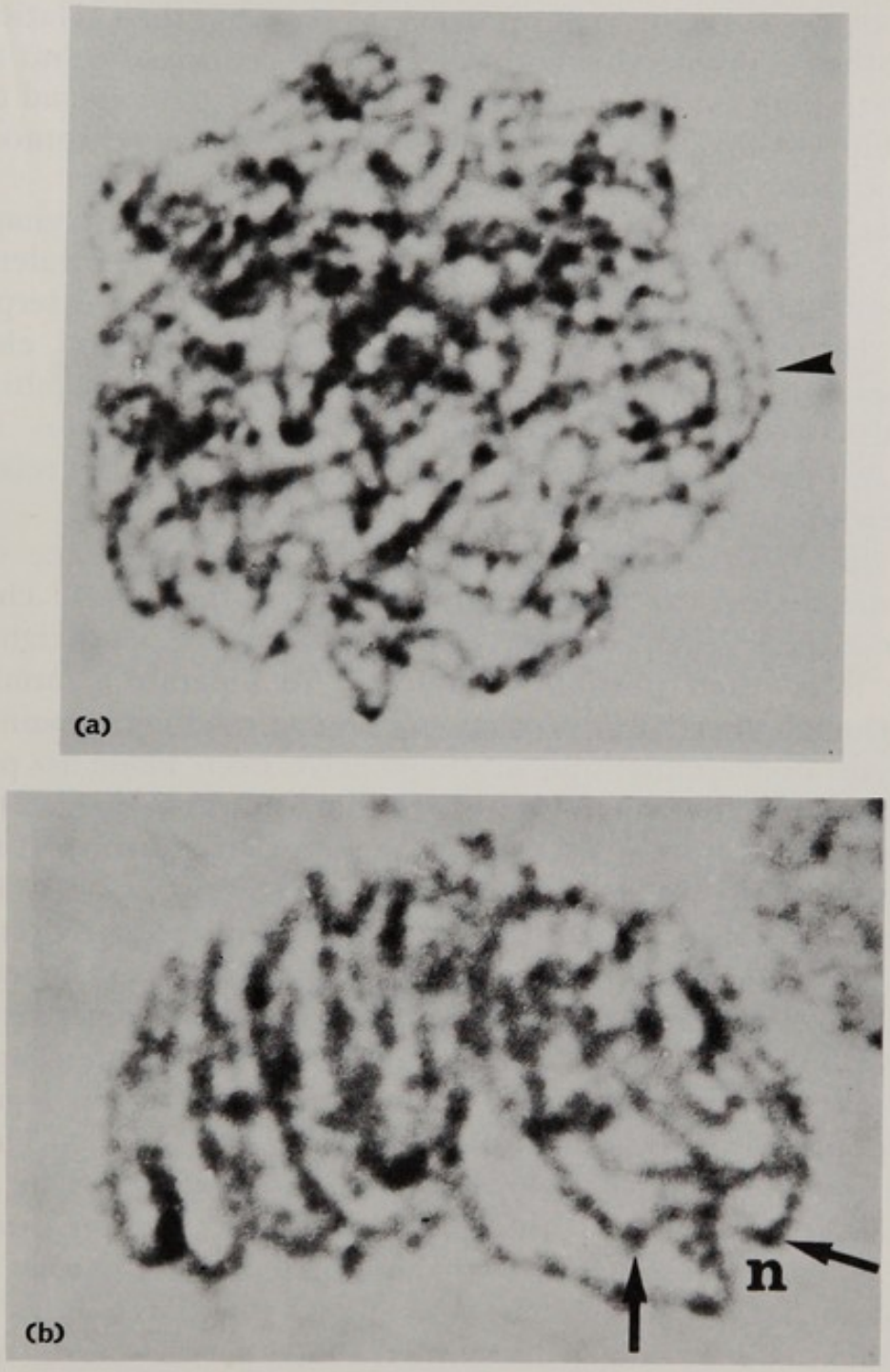


Fig. 20. (a) Leptotene, human primary spermatocyte. Chromomeres (arrowed) can be seen on the thread-like chromosomes. x2240. (b) Zygotene, human primary spermatocyte. The nucleolus (n) can be seen in contact with two terminal chromomeres (arrowed). x3250. Both reproduced by courtesy of Dr J.-M. Luciani.

possibly for a very short part of their length) condense during zygotene to form a small, dark-staining heterochromatic body called the *sex vesicle*. It is not really possible to see what their relationship to each other is within this body, because of its opacity, but as will be seen later, all the evidence indicates that they have an end to end relationship within it. As zygotene proceeds, all the chromosomes continue to shorten and thicken.

(iii) Pachytene (pachynema) (Fig. 21a and b). When synapsis is complete the cell has reached the pachytene stage. The bivalents are shorter and thicker than before (rather resembling furry caterpillars) and can be seen even more clearly, both on light and electron microscopic preparations, to be attached to the nuclear membrane at both ends (Woollam *et al.*, 1966, 1967). The nucleolus is still present, and the sex vesicle appears as a small dense body related to the nuclear membrane.

It is still difficult, because of the continuing presence of the nuclear membrane, to make a preparation of pachytene chromosomes in which each bivalent is separate from its neighbours. However it is often possible completely to separate a number of them, although there always seem to be some which remain stuck together or overlapping; and attempts have been made to prepare karyotypes of this stage, with limited success (Fig. 22). The pachytene chromosomes have more apparent structure than is seen earlier in meiosis. In good preparations the central parts of the bivalents can be seen to consist of two parallel threads (representing the two constituent chromosomes) on which there are a series of dark-staining enlargements, the *chromomeres,* strung together rather like the beads on a necklace, with thin lightly-staining areas in between them. Surrounding the twin threads is a fuzzy region, in which loops of chromatin can occasionally be seen. This has suggested to a number of workers (e.g. Baker and Franchi, 1967) that the chromosomes at this stage resemble, in miniature, the "lampbrush" chromosomes seen in amphibian oocytes (*q.v.*).

In the meiotic chromosomes of many plants, such as maize (McClintock, 1945) the chromomeres are much more obvious features, and can easily be seen in the leptotene chromosomes before synapsis; they are so distinctive in both size and position that they have made it possible to identify individual chromosomes at this and later stages, and to determine where certain genetic loci lie in relation to the chromomeres. This plant work has encouraged mammalian cytogeneticists to attempt to identify meiotic bivalents by their chromomere pattern. Early attempts in man were made by Schultz

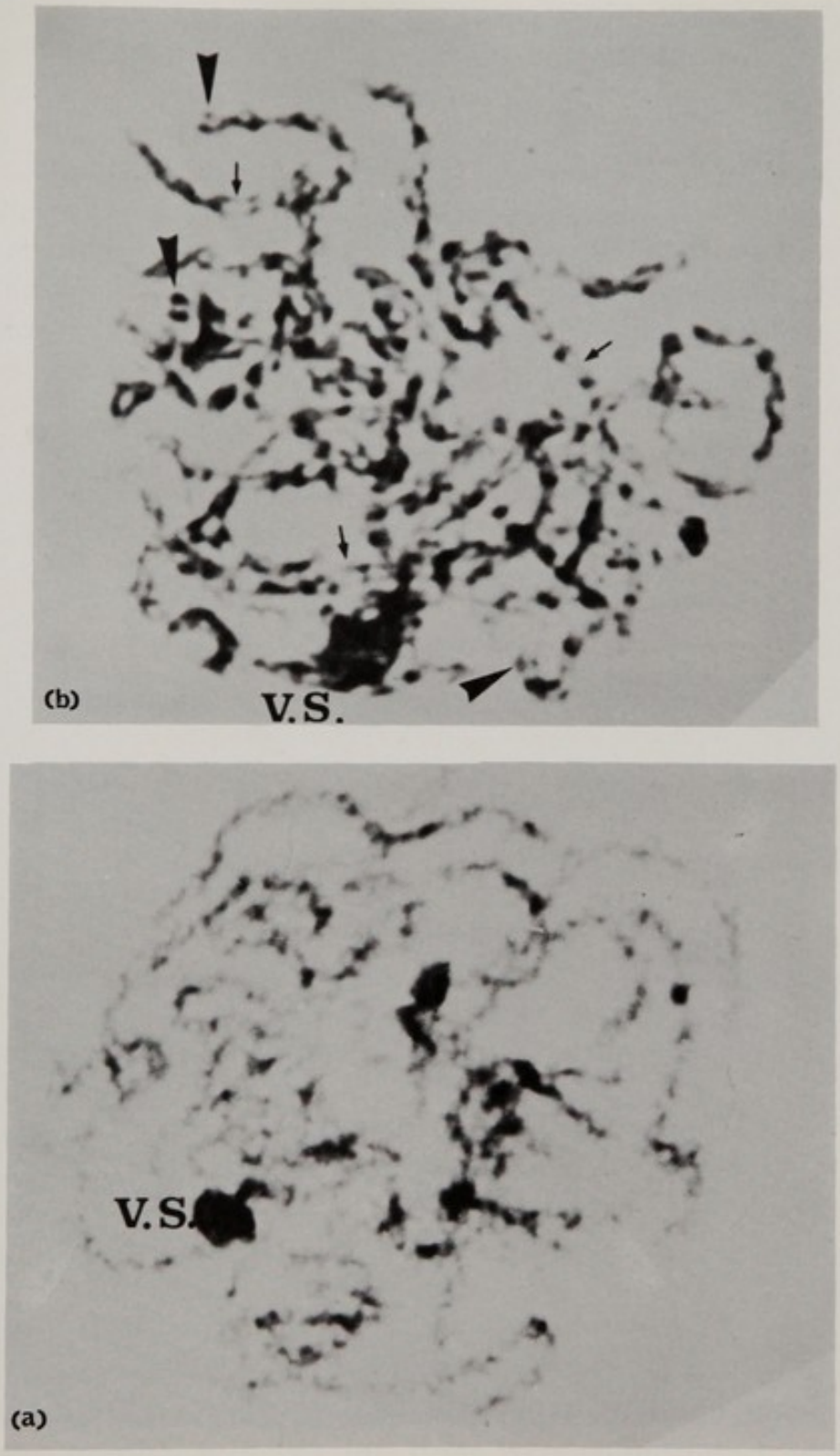


Fig. 21. (a) Early pachytene, human primary spermatocyte. The fluffy appearance of the chromosomes is reminiscent of the appearance of lampbrush chromosomes. x2200. (b) Late pachytene, human primary spermatocyte. Pairing of homologous chromomeres can be seen (heavy arrows). The small arrows indicate points where homologues are beginning to separate. v.s. = sex vesicle. x2360. Both reproduced by courtesy of Dr J.-M. Luciani.

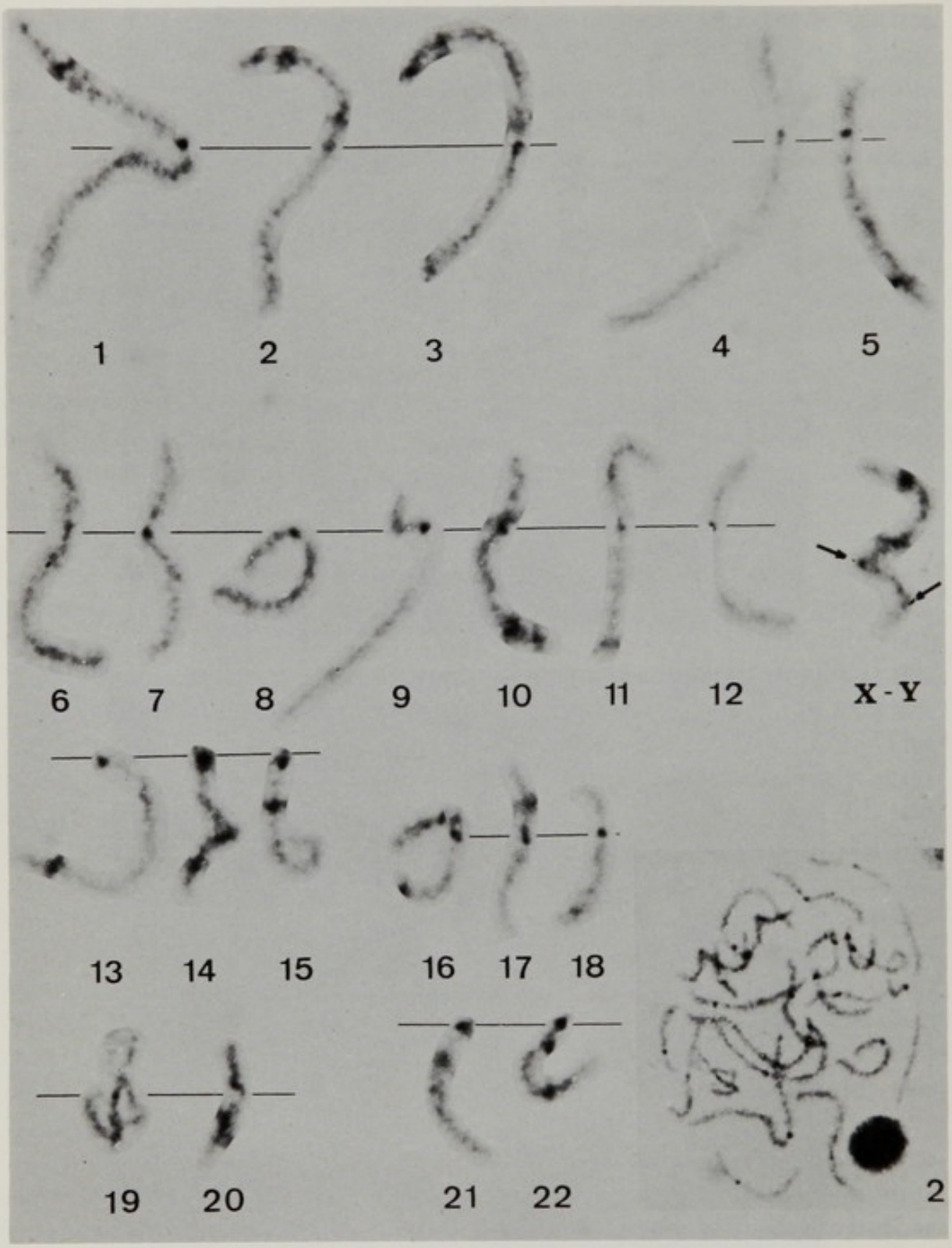


Fig. 22. Pachytene, human primary spermatocyte, with karyotype (Giemsa stain; the presumptive centromeres show up as dark spots). The arrows indicate the centromeres in the XY bivalent. Reproduced by courtesy of Dr J.-M. Luciani.

and St Lawrence (1949) and Yerganian (1957). However the much smaller size of mammalian bivalents in comparison to those of plants, and the difficulty involved in separating them completely from one another, has meant that although a number of bivalents have been successfully identified in individual preparations, no satisfactory chromomere map has yet been produced for all the human pachytene bivalents. However Hungerford and his coworkers (Hungerford, 1971; Hungerford *et al.*, 1971) and Bordjadze and Prokofieva-Belgovskaya (1971) have recently succeeded in making a tentative map of chromosomes 21 and 22 and have shown that these two chromosomes can be distinguished by their chromomere pattern. They have also been able to confirm by this method the findings which have emerged from autoradiographic and fluorescent studies of mitotic chromosomes (Chapter 2) to the effect that the chromosome which is trisomic in Down's syndrome is the shorter of the two (Hungerford *et al.*, 1970).

It is possible by Giemsa staining to show up a dark region in pachytene chromosomes which either represents the centromere, or possibly juxtacentromeric heterochromatin (Chen and Falek, 1969), and by using this method in conjunction with other criteria for chromosome identification such as size or association with a nucleolus, a good proportion of individual bivalents should be recognizable at pachytene (Fig. 22).

The association of pachytene bivalents with the nucleolus (Fig. 23a and b) in man was first described by Schultz and St Lawrence (1949). They described a "nucleolar chromosome" which seemed to be a large bivalent associated with the nucleolus at its centre. Ferguson-Smith and Handmaker (1961) consider that this configuration actually represents two acrocentric chromosomes associated with the nucleolus by their ends, and subsequent workers have supported this interpretation (Fig. 23b). Ferguson-Smith (1964) found up to five bivalents terminally associated with the nucleolus, and interpreted this as meaning that these five bivalents were the acrocentric chromosomes of groups D and G which take part, in mitosis, in the process of satellite association. Luciani (1968) was unable to find as many as five nucleolus-associated bivalents; the commonest number was two, but three were sometimes found. These figures might correlate with the variation in the number of satellited acrocentric chromosomes which can be seen in different individuals; but it remains to be proven that there is in fact a relationship between the number of satellited chromosomes seen in mitotic cells and the number of nucleolus-associated bivalents at pachytene.

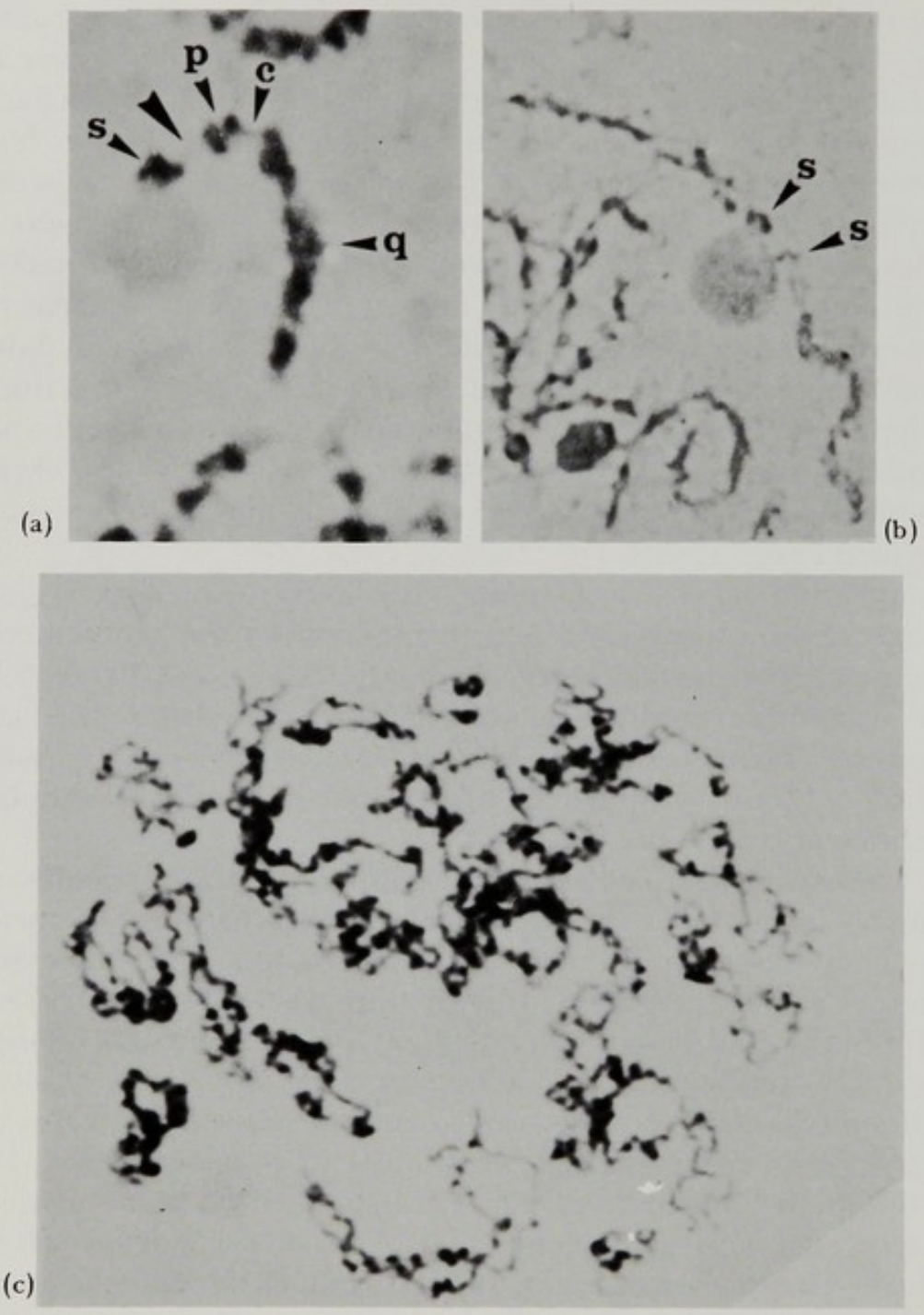


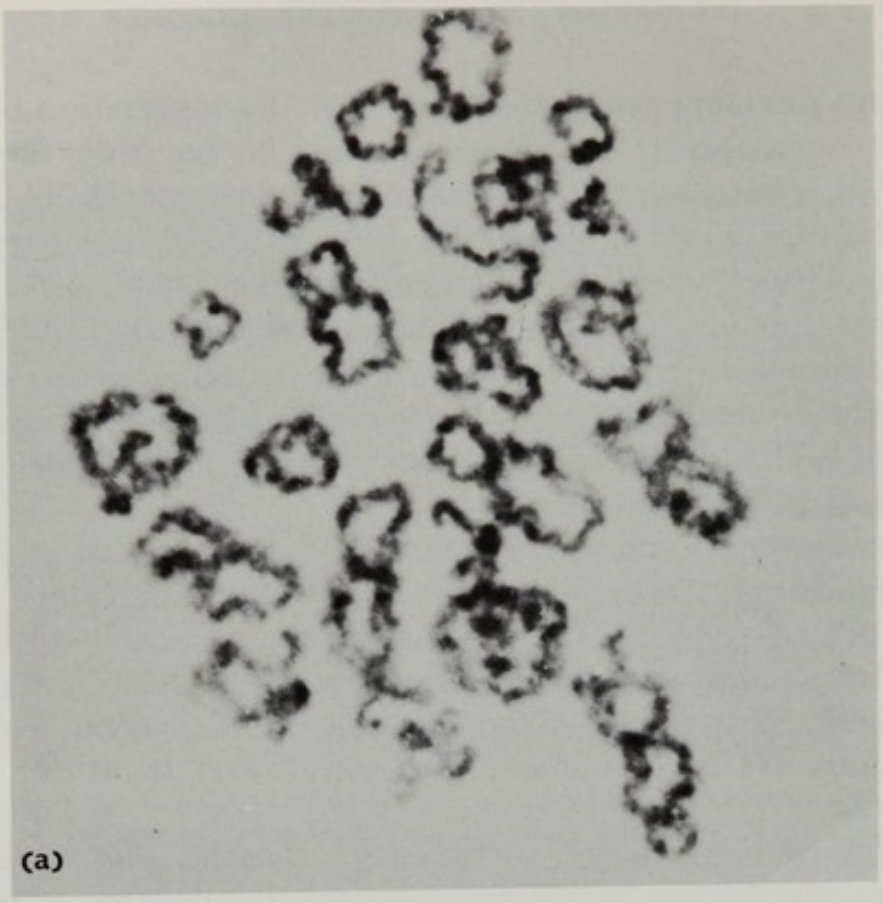
Fig. 23. (a) Terminal association of an acrocentric chromosome with the nucleolus, at pachytene. s = satellite, large arrow—secondary constriction, p = short arm, c = centromere, q = long arm. (b) Terminal association of two acrocentric chromosomes with the nucleolus. s,s = satellites. (c) Diplotene, human primary spermatocyte. Homologous chromosomes of the bivalents are falling apart, and chiasmata are visible. ×1920. Reproduced by courtesy of Dr J.-M. Luciani.

Another puzzling phenomenon is that the association of bivalents with the nucleolus at pachytene appears to be truly terminal; the association is between the satellite itself and the nucleolus (Fig. 23a), rather than between the associated constriction and the nucleolus (Luciani, 1968) which is difficult to reconcile with the well-established concept (founded on plant chromosome studies) that it is the secondary constrictions which are the actual nucleolar organizers. Luciani states that association of secondary constrictions with the nucleolus such as is seen in plant chromosomes is never seen in human meiotic preparations.

Besides the main nucleolus which is constantly seen at pachytene, Ferguson-Smith (1964) and Luciani (1968) have found a second, smaller one in 20-30% of cells, and occasionally a third. Unfortunately these observations were confined to a fairly small number of individuals, and it is quite likely that the number of nucleoli per cell, like the number of visible satellites, may differ from one person to another.

Hungerford *et al.* (1971) regularly observed one other curious feature in one of the group C chromosome bivalents at pachytene. This was a small ring of dark-staining bodies related to the long arm of the C bivalent. In Fig. 36 there is shown what is probably a section through this structure, as seen on electron microscopy. Its significance is at present obscure.

(iv) Diplotene (Fig. 23c) and diakinesis (Fig. 24). These, the last two stages of first meiotic prophase, are conveniently considered together since the distinction between them is not absolute. Swanson (1963) states that diakinesis is characterized by a more contracted state of the chromosomes, by the disappearance or detachment of the nucleolus from its associated bivalents, and by the even distribution of the bivalents throughout the nucleus. While these distinctions can be made clearly in some human cells, there are intermediate stages which are hard to categorize, particularly in squashed or air-dried preparations. *Diplotene* is characterized by the falling apart of the two chromosomes of the bivalents. Separation is not complete; the two chromosomes remain attached at certain points, the *chiasmata,* which are believed to represent the sites of genetic crossing-over. At these points there is an actual physical exchange of material between the maternally and paternally derived chromosomes (Taylor, 1965). The number of chiasmata present can be counted in cells from diplotene through to metaphase. The mean number, according to several authors, is between 44 and 58, with a range of 35-66 (C. E. Ford and Hamerton, 1956; Böök and Kjessler,



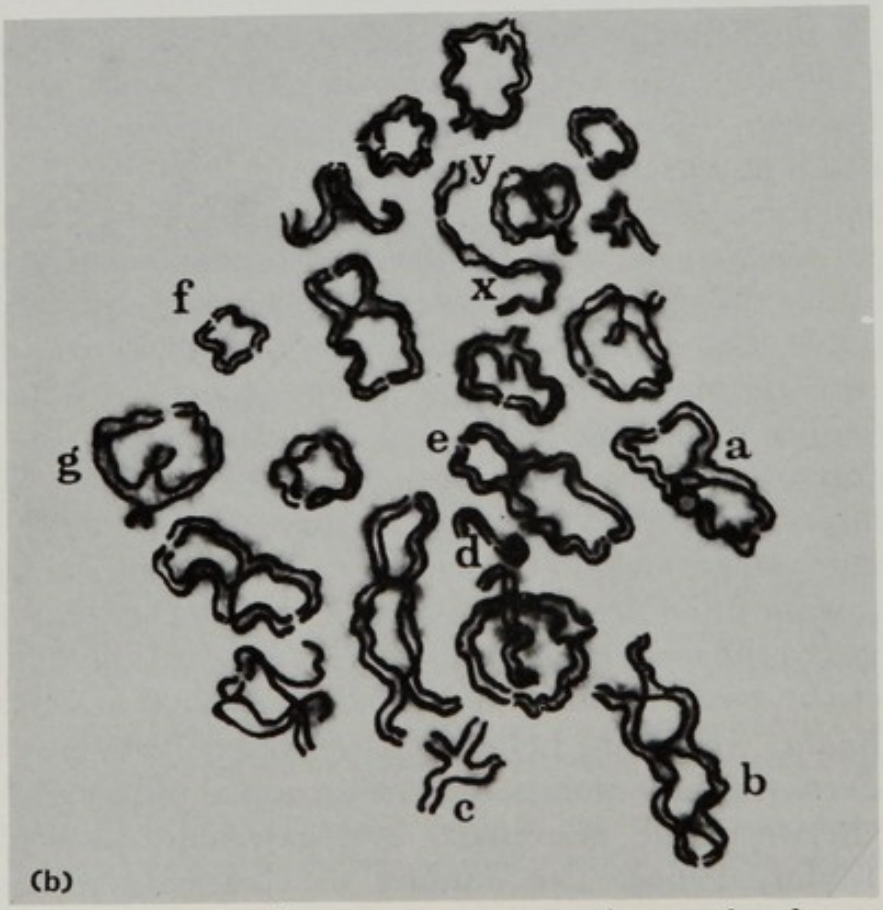


Fig. 24. Diakinesis, human primary spermatocyte, to show number, frequency and type of chiasmata present. The lower figure (b) gives the interpretation of the chiasmata seen in the upper figure (a). Forty-seven chiasmata are present. a,e—Bivalents with one interstitial and two terminal chiasmata; b—Bivalent with three interstitial and one terminal chiasmata;

1964; Sasaki and Makino, 1965; MacIlree *et al.*, 1966; Eberle, 1966; Luciani, 1968). Kjessler (1966) found that 16.2% of bivalents had one chiasma, 39.7% had two, 33.4% had three, 9.6% had four, 1% had five, and occasional bivalents with six chiasmata were seen. The number of chiasmata per bivalent is related to length. The cross-overs which occur at the chiasmata ensure the redistribution of the genetic material derived from the individual's parents in such a way that each gamete produced has a unique genotype.

The sex chromosomes behave differently from the autosomes at diplotene. The sex vesicle disappears, and the X and Y chromosomes can be seen to be associated end to end (Fig. 24). No chiasma can ever actually be seen between them, although some workers have considered that a small one is (or should be) present. The association is considered in more detail later when the sex vesicle is further discussed.

As diplotene progresses to *diakinesis* the chromosomes progressively shorten and thicken, and the chiasmata are traditionally considered to *terminalize*; to move towards the ends of the chromosomes. However there are some who consider that the terminalization of chiasmata is an artifact due to preparation. The nucleolus progressively disappears, and the nuclear membrane begins to dissolve.

(c) First metaphase (Fig. 25). As metaphase approaches, the spindle begins to form, just as at mitotic metaphase, and the nuclear membrane disappears completely. The bivalents then form up on the equator of the cell to form the metaphase plate. There is a distinctive difference from mitotic metaphase in the behaviour of the centromeres at this stage. Whereas in mitosis the centromere is at first undivided and lies in the plane of the metaphase plate, the centromeres of the homologous chromosomes forming a bivalent at first meiotic metaphase remain separate, and because of their attachment to the spindle they lie on either side of the equator of the cell.

The form of the individual metaphase bivalents is related to the number of chiasmata which they possess, and to the terminalization of these. Where there is only one chiasma, a cross-shaped structure is formed at diakinesis, which proceeds to form a rod-shaped structure at metaphase, when the chiasma terminalizes. Two chiasmata, when

---

Fig. 24, *continued*

c—Bivalent with a single chiasma; f—Ring bivalent with two terminal chiasmata; g—Ring bivalent with one terminal chiasma and one not yet terminalized. Reproduced by courtesy of Dr J.-M. Luciani.

Fig. 25. First meiotic metaphase, human primary spermatocyte. The forms of the bivalents resemble those seen at diakinesis, but further contraction has occurred.

terminalized, will give a ring-shaped structure at metaphase; two semicircular chromosomes associated at their ends. Three chiasmata will give a figure-of-eight (since the central chiasma cannot terminalize as readily as the others) and four or more chiasmata will add additional loops to this configuration. The number of chiasmata can be counted by examining these forms in the whole set of bivalents; there is one difficulty, which is that it may be difficult to tell a central chiasma in a long bivalent apart from a simple crossing of the two chromosomes due to the preparation.

The X and Y chromosomes remain associated end to end at metaphase, although in many cells (up to 40% according to some observers) they appear separated, as *univalents*, so that 24 separate

bodies may be seen in the cell instead of 23. However this separation may be related to the technique of preparation, rather than to an actual separation *in vivo*. The former interpretation is favoured by the observation that separation is much more frequently seen in air-dried preparations, where the mechanical spreading effect is greater than in squash preparations.

(d) First meiotic anaphase. The whole chromosomes move rapidly towards the two poles of the spindle. This is a stage which because of its short duration is not often seen.

(e) Telophase. At telophase the haploid chromosome complement is now present in each of the two daughter cells, the *secondary spermatocytes*; the nuclear membrane reforms, and the reduction division is complete. However this stage is never completed in the same way as mitotic telophase; the daughter cells remain connected by a cytoplasmic bridge, as described earlier, and the chromosomes do not disappear, but remain as dark rods attached to the nuclear membrane until the commencement of the second meiotic division, which probably takes place very shortly after the first is completed.

(f) Second meiotic division. The cell now passes, without any effective prophase, into the second meiotic metaphase (Fig. 26). The nuclear membrane dissolves, and the spindle reforms. The 23 chromosomes of the haploid set can be seen aligned on the spindle (or, in the usual preparation, spread upon the slide). Each one displays its two chromatids, joined at the centromere. The morphology of the chromatids is rather reminiscent of that of the chromosomes at spermatogonial metaphase; they are coarsely spiralized, and rather fluffy in appearance. At *second anaphase* the two chromatids constituting each chromosome separate at the centromere and pass to opposite poles; thus at telophase the daughter cells, the *spermatids,* each contain one complete set of genetic material. This represents one quarter of the material present in the primary spermatocyte from which they originated, and is derived approximately equally from each parent of the individual concerned, and assorted (apart from the X and Y chromosomes, which probably pass intact from one or the other parent) in a random fashion within each chromosome, due to the crossing-over which has taken place.

(g) Spermiogenesis. After the second meiotic division is complete the spermatids undergo a complicated series of changes (during which they are infolded in, and presumably nourished by, the Sertoli cells), and become converted into spermatozoa. Since these changes are mainly concerned with the cytoplasmic parts of the cell, and with

Fig. 26. Second meiotic metaphase, with karyotype. The coarse spiralization of the chromatids of the haploid chromosome complement is well shown; association is visible between two acrocentric chromosomes of groups D and G (arrowed). Reproduced by courtesy of Dr J.-M. Luciani.

the conversion of the immobile spermatid into a motile spermatozoon, they will not be further considered here except inasmuch as they concern the nucleus and the contained genetic material. The small spermatid nucleus becomes even more condensed, so that when the spermatozoon is complete its head, formed largely from the nucleus, appears to be composed of almost solid chromatin. It is very densely staining, and even on electron microscopy no organized structure can be seen in sections, although fibrils can be shown to be

present in preparations made by the modified Gall (whole chromosome) method. Bianchi and De Bianchi (1969) have demonstrated that in the rat the Y chromosome appears (as shown by autoradiography) to occupy a definite and constant position in the sperm head. They suggest that the other chromosomes are arranged end to end in the sperm head in an ordered manner. Since the Y chromosome appears to occupy a band transversely across the middle of the sperm head, it seems unlikely that the chromosomes are lying in a longitudinal parallel array.

4. The Timing of Male Meiosis

Heller and Clermont (1964) have studied the germinal epithelium of the human testis by autoradiography, and have been able to estimate the time taken by cells to evolve from spermatogonium to spermatozoon. The actual cytological findings are complex, but Heller and Clermont found six typical patterns of cell association in different areas of tubules. They have interpreted this, in conjunction with their autoradiographic findings, as showing that the seminiferous epithelium passes through a succession of cycles of activity; each of these lasts about 16 days, and during it the epithelium passes through the six stages already mentioned. 3.8 consecutive cycles occur in any region of the epithelium before a particular cell has passed through its whole evolution from spermatogonium to spermatid, and the whole process takes on average about 61 days.

Several observers have considered that spermatogenesis occurs in waves which pass down the seminiferous tubules from one end to the other, but this is probably not so, and the actual process is one of cyclical activity going on independently in different regions of a single tubule.

Burgos and Fawcett (1955) and Fawcett *et al.* (1959) have described how intercellular bridges can be demonstrated by electron microscopy between groups of spermatocytes and between spermatids, in such diverse species as *Hydra, Drosophila* and man (Fig. 27). These bridges appear to be of universal occurrence and probably result from incomplete separation of the spermatocytes at spermatogonial, first and second meiotic telophase. Thus all the cells which have been derived from a single spermatogonium are probably conjoined in this way. So it is often seen that primary spermatocytes occur in conjoined pairs, secondary spermatocytes in conjoined fours, and spermatids in conjoined groups of eight (or even 16). This syncytial development accounts for the synchronous development of these groups of cells, and also for the way in which they are often

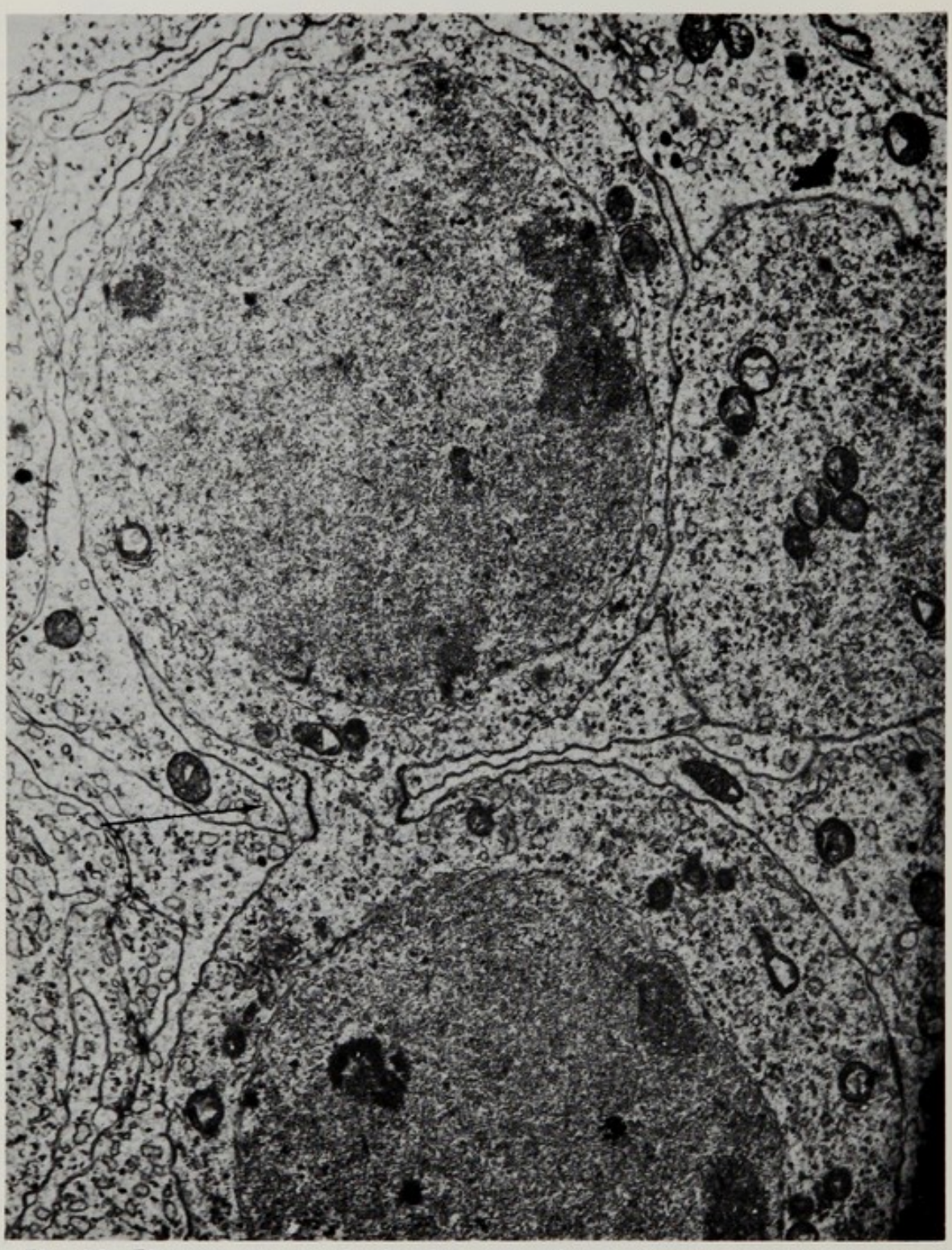


Fig. 27. Electron micrograph showing two primary spermatocytes (of a mouse) connected by an intercellular bridge (arrowed). ×12,000.

found in groups in meiotic chromosome preparations and sometimes form polyploid cells at first or second meiotic metaphase. Multinucleate cells are often found in smears or teased preparations of testicular tissue, though they are not often seen in histological preparations.

The various stages of meiosis differ considerably in length, so that as might be expected, the proportion of cells found at different stages in meiotic preparations varies considerably. First meiotic prophase is a long stage (primary spermatocytes have a life of over 3 weeks); within it, leptotene is fairly long, and reasonably easy to find in preparations; zygotene is more transitory, and less easy to find. Pachytene is a long stage, and cells at this stage form a majority of those seen. Diplotene and diakinesis are again fairly transitory, and fairly few cells are found in these stages. Metaphase is not usually hard to find, but is often confined to certain areas of tubule; it will not be found anywhere, like pachytene. First anaphase is very short and hard to find. Second metaphase is also fairly short, and not easy to find. But if the right area of tubule is located, clusters of cells may be found in any of these stages.

5. The Sex Vesicle and the Problem of Partial Sex-linkage

The term *sex vesicle* was coined by Sachs (1955) to describe the body formed by the X and Y chromosomes at the end of leptotene, which lasts until diakinesis. This body, which is probably formed by precocious condensation of the two chromosomes, after which they may be covered by an envelope of RNA, effectively obscures the behaviour of the sex chromosomes while they are within it. However, careful cytological studies just before the vesicle is formed, and just after its dissolution, indicate that the sex chromosomes are associated end to end within it; and this is certainly the relationship which they bear to each other at metaphase.

It was at one time considered extremely likely that the X and Y chromosomes of man possessed homologous segments; segments containing homologous gene sequences which might be expected to pair at meiosis and to form chiasmata. Against this theoretical background, search was made for genetic loci in man which might show "partial sex-linkage", that is, might exist on these homologous segments. Detection of such loci is difficult (because their behaviour is so similar to that of autosomal gene loci) and involves complex mathematical considerations, but Haldane (1936) was able to propose a list of genes which appeared likely to be partially sex-linked. This list has not stood up to critical examination, and cytogenetic studies to date have afforded no clear evidence for homologous segments in the X and Y chromosomes which participate in chiasma formation; all that has ever been seen with certainty is an end to end association. The cytological evidence is discussed further (in relation to fine structure) in the section on the fine structure of meiotic chromosomes (Chapter 6).

It is not really possible in ordinary meiotic preparations to discern which arms of the two sex chromosomes are involved in this end to end association. If partially sex-linked genes exist, it would be of considerable interest to know this (less so if they do not). Recent studies using the Giemsa and fluorochrome staining techniques (Chen and Falek, 1969; Pearson *et al.*, 1970) seem to have proved that it is the short arms of each of the two chromosomes which take part in their terminal association.

B. Meiosis in Women

1. Development of the Ovary, and Timing of the Meiotic Cell Divisions

The various stages of the two meiotic divisions in women are cytologically comparable to those in men, but differ considerably in detail, mainly because of the profound difference in timing of the whole process. Whereas in men the spermatogonia only start to divide rapidly and to differentiate into spermatocytes at the age of puberty, and having once started to differentiate proceed through the whole cycle from spermatogonia to spermatozoa in 8-9 weeks, the comparable cells in women, the *oogonia,* divide and differentiate into *primary oocytes* before birth; but the first meiotic division is not completed in any particular oocyte until shortly before that oocyte is shed at ovulation, an event which may take place anything from 10 to 50 years later.

Early development of the ovary is rather similar to that of the testis. The germ cells migrate into the gonad, from their original position around the stalk of the yolk sac. Even before the gonad differentiates into a recognizable ovary, the female germ cells tend to be concentrated in the cortical (outer) region, in contrast to the condition in male embryos where they are more concentrated in the medulla or central region (Mintz, 1960). The subsequent changes have been described by Ohno *et al.* (1962). In a 3-month fetus the ovarian cortex is composed of "ovigerous cord" containing a loose mixture of oogonia, developed from the germ cells, and of follicular cells (the mesodermal cells whose future function is to surround and nourish the oocyte). Many mitoses are seen, and in the deepest layer of the cortex a few oogonia have differentiated into primary oocytes and entered into the leptotene stage of first meiotic prophase, occasionally progressing as far as zygotene. In a 4-month fetus the fetal ovary has a similar appearance, but large numbers of cells can

now be seen in the deepest layers of the cortex which have progressed to leptotene and zygotene. The appearance of the primary oocytes at this stage is very similar to that of primary spermatocytes, except that the cells are somewhat larger; and throughout the development of the oocytes these cells continue to enlarge, so that both the nuclear and the cytoplasmic components of the cell eventually become very much larger than those of spermatocytes, particularly the latter. This is because when these cells are eventually fertilized and develop into an embryo, that embryo will be very dependent, in its earliest preimplantation stages, on an adequate store of nutrients within the cell.

In the 7-month fetus all the oogonia have disappeared; they have all differentiated into oocytes. In the ovarian cortex cells in leptotene or zygotene are loosely mixed with follicular cells; in the deeper two thirds of the cortex each oocyte is surrounded by a single compact layer of follicular cells, forming a *primordial follicle.* The oocytes in these follicles are at the pachytene or diplotene stages of first meiotic prophase.

In the testis, diplotene heralds the end of first meiotic prophase; the chromosomes shorten and pass through diakinesis to metaphase; but in the ovary the diplotene bivalents do not condense, but instead become longer and finer, and less distinct from each other. They are entering a resting phase called *dictyotene* (the dictyate stage), in which they remain until shortly before ovulation, many years later. In sections the dictyate chromosomes are so indistinct that the nucleus looks almost like an interphase nucleus, but squash preparations reveal that the nuclei still contain modified diplotene bivalents, which are in a very extended state. Baker and Franchi (1967) have described the structure of the human oocyte during the "resting phase" after birth. The chromosomes at this stage resemble the "lampbrush chromosomes" of amphibian oocytes (*q.v.*) in that they have a visible core surrounded by loops of coiled fibrils, which form symmetrical structures on either side of the core. In the rat the dictyate and diplotene stages can be distinguished with the electron microscope; at diplotene a definite "core" can be seen, while at the dictyate stage no cores can be seen, only a random granulation, presumably because of the more extended state of the chromosomes (Franchi and Mandl, 1962). Human postnatal oocyte chromosomes resemble those of the diplotene stage in the rat, in that they possess visible cores. Baker and Franchi (1967) have noted that rat and mouse oocytes are more radiosensitive at this stage than are human oocytes, and attribute this to their structure, and especially to the

greater extension of the lateral loops coming off the central areas of the chromosomes; these have a protective sheath of RNA and protein, and it seems likely that the more attenuated this becomes, the more vulnerable are the chromosomes to ionizing radiations.

After birth the oocytes remain in the resting dictyate stage, but slowly and progressively enlarge, for reasons which have already been given. The cytoplasm fills with yolk, and the enlargement of the nucleus is presumably related to its activity in inducing the protein synthesis which is necessary to this storing of nutrient. The follicular cells surrounding the oocyte multiply, and eventually become several layers thick.

At puberty, under the influence of pituitary gonadotrophic hormones (follicle-stimulating hormone, FSH) certain of the ovarian follicles enlarge dramatically each month. Follicular fluid, which already separates the follicular cells into two layers, a peripheral one and one surrounding the oocyte ("zona radiata"), increases enormously in volume in these follicles. In the oocytes themselves the elongated bivalents quickly condense, chiasmata terminalize, and the chromosomes form up on the first meiotic metaphase plate. At metaphase the homologues separate, as in the male, and the reduction division is achieved. However because the dictyotene nucleus is eccentrically placed (and also the axis of the developing spindle), when the oocyte divides one of the two daughter cells receives almost all of the cytoplasm. This cell is the *secondary oocyte* and the other daughter cell, which consists of little except nucleus, is the *first polar body*. Ovulation takes place at about this time; the fluid-filled follicle, which is at the surface of the ovary, ruptures and the oocyte is extruded, surrounded by follicular cells ("corona radiata"). Usually only one of several oocytes which have developed in any particular month is extruded, but occasionally more than one is thrown out, both of which may be fertilized and thus give rise to dizygotic (dissimilar) twins.

After the secondary oocyte, accompanied by the first polar body, pass to the Fallopian tube, both nuclei proceed fairly rapidly to second meiotic metaphase. The second meiotic division is not normally completed, however, until the time of fertilization, if this takes place. When the division occurs cytoplasmic division is again unequal, and a second polar body is extruded. The first polar body may itself divide so that the fertilized egg may be surrounded by three polar bodies at this stage.

It is almost impossible under normal circumstances, for obvious technical reasons, to study the cytogenetics of the period round

about ovulation. However Edwards has recently devised a technique which has made study of the female meiotic divisions, fertilization, and even early development of the embryo (cleavage) possible (reviewed in Edwards and Fowler, 1970). This technique is based on the discovery that if follicle-stimulating hormone (FSH) is injected into normal female animals or women it can produce simultaneous maturation of a large number of ovarian follicles (up to 30 or 40). These shed their oocytes on to the surface of the ovary, and these oocytes can be collected by means of a peritoneoscope (a sort of telescope which is inserted into the peritoneal cavity through the umbilicus; Steptoe and Edwards, 1970). The further development of the oocytes can then be studied *in vitro* in a suitable culture medium. As previously mentioned, *in vitro* fertilization has now been achieved.

2. Cytogenetics of Oogenesis

The appearance of the chromosomes during the first part of first meiotic prophase has already been described. The appearance of the chromosomes at these stages is very similar to those in men; the main differences in cytology lie in the greater size of the oocytes, and in the difference in the time scale of development. The first real cytogenetic difference is in the presence in the oocyte of the additional dictyotene stage of meiotic prophase, which is a modified diplotene stage, lasting many years, and during which the chromosomes are capable of considerable activity in protein synthesis, and are therefore in a more extended state than in the comparable male diplotene stage. Another difference is that the sex chromosomes are, of course, homologues, and behave in a similar fashion to the autosomes, from which they cannot be distinguished. They display no precocious heterochromatinization, nor do they participate in the formation of a sex vesicle.

The fact that the synaptic process in women starts before birth means that meiotic studies of the sort that have been carried out on the testis, and which have given information on such topics as the detection of chromosomal aberrations in cases of infertility, are very difficult to perform in females. Study of the actual first and second meiotic divisions is now possible by the technique of R. G. Edwards, but while this is of considerable fundamental interest it remains, so far as clinical medicine is concerned, very much a research technique. However photographs of the female meiotic chromosomes at first metaphase have been published (Yuncken, 1968; Edwards and Fowler, 1970) and preliminary estimates of chiasma frequency have

been made. These suggest that the frequency may be comparable to but perhaps slightly lower than that found in spermatocytes (Edwards and Fowler, 1970), although Yuncken (1968) found 52 in one oocyte at diakinesis. There is evidence in the mouse that chiasma frequency in the female mouse decreases with age, and it has been suggested that this might be responsible for an increase in *non-disjunction* (*q.v.*) with increasing age in both mice and women (Henderson and Edwards, 1968). However decreasing chiasma frequency with increasing age has not yet been satisfactorily demonstrated in women (see Chapter 11).

III. THE CLINICAL SIGNIFICANCE OF MEIOTIC STUDIES ON THE TESTIS

Meiotic studies have one great advantage over studies of mitotic chromosomes. This is due to the fact that during meiosis homologous chromosomes synapse with each other; and it appears that a certain homology of linear structure must exist for synapsis to take place. The phrase "a certain homology" is used advisedly, since it is clear that homologues cannot be absolutely identical in their basic DNA structure. However it is evident from studies on such favourable material as plant or *Drosophila* chromosomes that where chromosomal rearrangement has taken place in one of a pair of homologues, this is reflected in abnormal pairing, or sometimes absence of pairing, at meiosis. The underlying principle seems to be that homologous regions of chromosomes, even if they are displaced (by inversion or translocation to another chromosome, etc.) must pair with each other, and in the same linear direction. It follows that such displacement will give rise to various visible anomalies in the chromosomal bivalents after synapsis (Chapter 8). If two supposed homologues are very far from homologous (as may occur in the hybridization of two related but differing species of animals, such as zebra and donkey) then they do not pair but form *univalents.* In contrast to this, reciprocal translocations between chromosomes will result in complex configurations at meiosis, due to the joining together of three, four or even more chromosomes to form *trivalents, quadrivalents* and so on, which will appear in the form of rings or chains.

Thus the recognition of abnormal configurations of the chromosomes after they have synapsed can give information about linear changes in their structure which cannot necessarily be seen in mitotic

chromosomes. Whether this alteration in pairing behaviour is directly related to an alteration in DNA sequence or to some coarser order of structural differentiation cannot be determined on the information available at present.

A number of studies on the male human meiotic chromosomes have been carried out, and have been reviewed by Kjessler (1970). These have mainly been concerned with the chromosomes at first meiotic metaphase and at the immediately preceding stages (diplotene and diakinesis) since it is at these stages that abnormal synapsis is most readily studied. There have been several descriptions of the meiotic chromosomes in cases of autosomal structural heterozygosity. Trivalents have been seen in patients with known autosomal translocations (such as D/G and D/D translocations), quadrivalents have been seen in other translocations (C/C, 15/18), and failure of pairing has been observed in homologues when one of the pair appeared in mitotic cells as a ring chromosome (McIlree *et al.*, 1966). Such configurations have also been seen in subfertile patients with apparently normal mitotic chromosomes. This may indicate that these patients possess a hidden reciprocal translocation or other cause of structural chromosomal heterozygosity.

Studies have also been performed on patients with trisomy 21, Down's syndrome. There are no known instances where a male patient with Down's syndrome has had offspring. Nevertheless, spermatogenesis has been found to occur in some of these patients (Kjessler, 1970). It is of considerable interest to know whether the three chromosomes 21 form a trivalent at synapsis, or whether two of them form a bivalent and the other remains unpaired. Either condition is theoretically possible. Miller *et al.* (1960) and Hungerford *et al.* (1970) found trivalent formation (as well as formation of bivalents and univalents) in patients with Down's syndrome. Sasaki (1965) considered that there was evidence of trivalent configurations in another patient; but Finch *et al.* (1966) and Kjessler (1970) found no trivalents in one and two patients respectively.

McIlree *et al.* (1966) have described two cases of structural alterations of the Y chromosome studied at meiosis. In one, apparently a mosaic for a dicentric Y chromosome, the X and dicentric Y appeared as univalents in most cells. In another case with a metacentric Y chromosome (presumably due to a pericentric inversion) end to end pairing of X and Y was seen.

Cases of sex chromosome aneuploidy have also been studied. Sex chromosome aneuploidy does not necessarily imply a reduced fitness

to reproduce; this has not always been found in cases of the 47,XXX and 47,XYY syndromes which have been studied. 47,XXY individuals suffer, of course, from seminiferous tubule dysgenesis (Chapter 10) and are probably always sterile. It appears that in persons with sex chromosome aneuploidy there is selection against the aneuploid gametes produced during gametogenesis, so that the children of such persons are usually diploid. This applies particularly to 47,XXX women. No case of a 47,XXX woman having a child with sex chromosome aneuploidy has been described. However there are one or two instances of 47,XYY men with 47,XYY sons (Chapter 10). Thompson *et al.* (1967) studied meiosis in a 47,XYY man and found that not one of 155 spermatocytes examined clearly contained 2 Y chromosomes; this suggested that selection towards euploidy might take place before meiosis began. But there is no evidence as to what mechanism might be involved.

Kjessler has analysed meiosis in two men with sex chromosome mosaicism, who had both 46,XY and 45,X cell lines. In both cases a germ cell line with a 45,X karyotype was found. This cell line proceeded to diakinesis with a single sex chromosome and with no evidence of cell degeneration, implying that a viable gamete might have been produced which lacked a sex chromosome, and which could have fertilized an egg. Kjessler also studied a patient with 46,XY/47,XXY mosaicism and could find no cells of the 47,XXY line at first meiotic metaphase. He suggests (like Thompson *et al.* previously) that this could be due to a premeiotic selection mechanism operating against aneuploid spermatogonia.

It is perhaps rather early to try to estimate the significance of meiotic studies in clinical medicine when so few have yet been carried out. However it seems clear that they can be of value both because of the information they can give about gametogenesis in individuals with abnormal chromosomes, and perhaps more immediately as an aid in the diagnosis of the causes of infertility. As an instance of this, McIlree *et al.* (1966) found that in a group of 50 subfertile males examined (all chromatin-negative, and therefore excluding cases of Klinefelter's syndrome of known infertility), in only 28 of whom dividing cells could be analysed, five (10%) had a structural chromosome abnormality which was likely to be causally related to their reduced reproductive ability. This suggests that meiotic analysis should be carried out in any case in which a testicular biopsy is performed for the purpose of diagnosing the cause of infertility.

§ 4 §

Comments on Techniques for the Study of Chromosomes

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I. INTRODUCTION

There are several good books which are largely concerned with the techniques of human cytogenetics, and therefore it is not intended to deal with the details of technique here; but rather to discuss briefly the principles which are involved, and then to refer the reader to the sources which describe the most common and useful techniques which are in use.

Every laboratory and worker have their own modifications of the principal techniques; these modifications, although usually minor, add an element of confusion to the subject, and it is always best for a beginner to start by actually going to a laboratory which employs cytogenetic techniques, and to see how they are carried out. This will

always save time and trouble. In many of the accounts in books and papers, some small (even apparently trivial) stage is omitted or inadequately described.

The following works are recommended as major sources of information on cytogenetic techniques, in addition to original papers on the subject: "Human Chromosome Methodology" (J. J. Yunis, ed.), Academic Press, New York and London, 1965; "Human Cytogenetics" by John L. Hamerton, Vol. 1 ("General Cytogenetics"), Academic Press, New York and London, 1971; "Cytogenetics" by Jean H. Priest, Lea and Febiger, Philadelphia, 1969. These three works cover almost all the major techniques which are necessary, apart from the very recently described fluorochrome and Giemsa techniques for demonstrating heterochromatin (see Chapter 2), for which reference to the original sources is still necessary.

II. AVAILABLE SOURCES OF DIVIDING CELLS

A. Possible Sources

It is obvious that chromosome preparations for the light microscope can only be made from dividing cells (since the interphase chromosomes are too fine to be resolved), and that these cells must be at or near metaphase. This unfortunately limits the observer to the study of cells which are still capable of division. In the child or adult this effectively excludes all highly differentiated cells from study; such cells as neurones or those of striated muscle are inaccessible, and in practice, only rather simple and undifferentiated cells can be used. Those cells which have been used for the study of metaphase chromosomes in man fall into one of the following categories:

(a) Dividing cells in the bone marrow (precursors of the red and white blood corpuscles); in the embryo these cells may be found in other sites such as the liver and spleen.

(b) Lymphocytes of the peripheral blood; these do not probably divide in the bloodstream *in vivo*, but can nevertheless be induced to do so in culture.

(c) Unspecialized connective-tissue cells which can be grown in culture from a variety of tissues, and which fall into two main categories: fibroblasts (the most commonly studied), and wandering amoeboid cells of the macrophage type.

(d) Dividing cells in epithelia, such as those of the basal layer of the epidermis, which can be obtained in man from the root of a plucked hair (Daker, 1970). In mammals the cornea is a useful source of these cells, but it is not a practicable source in man. There should also be many dividing cells in the gut epithelium, but this is a source which is relatively inaccessible, and has not yet been exploited in man.

(e) Dividing cells in the gonad; in the testis both mitotic and meiotic cells are found (although the morphology of the chromosomes from spermatogonial metaphases is slightly different from that of chromosomes of somatic cells); in the ovary, cells are only available in the embryonic ovary (with the exception that meiotic cells can be obtained experimentally from adult women—see Chapter 3).

The extent to which these various tissues have been used in practice relates very much to the convenience with which they can be obtained.

B. Common Use of the Sources Available

The sources most commonly used will be considered briefly in order of importance and frequency of use.

1. Lymphocyte Culture

An enormous majority of human chromosome studies have been performed on dividing lymphocytes obtained by some modification of the method first described by Moorhead *et al.* (1960). This was based on the discovery that peripheral blood lymphocytes could be induced to divide by treatment with phytohaemagglutinin, a naturally-occurring substance prepared from beans, which was first used to separate the erythrocytes from the leucocytes because of its property of agglutinating erythrocytes. It was an extraordinary and very fortunate coincidence that this substance also happens to be strongly mitogenic (i.e. has the property to induce mitosis) to lymphocytes. On exposure to phytohaemagglutinin (PHA) in culture (or *in vivo*) the lymphocytes increase in size and start to divide in under 48 hours.

Other substances have since been shown to be mitogenic also, including pokeweed (PWM), tuberculin, antilymphocytic serum, various other antigens, and leucocytes from another individual (mixed lymphocyte reaction). The system whereby lymphocytes

may be induced to "transform" by various substances has proved a very potent research technique in many immunological experiments, apart from its use in cytogenetics, and has such a large literature of its own that it would be inappropriate to consider it further here.

The fact that lymphocyte culture has become the method of choice for most workers is simply due to its convenience. Blood is easily obtained (by venepuncture, or for "micromethods" by merely pricking a finger or earlobe); culture takes only 2-3 days and demands a minimum of equipment.

2. Fibroblast Culture

This has been widely practised in order to obtain chromosomes from a different type of cell. It has considerable disadvantages compared with lymphocyte culture. It demands a small biopsy (usually of skin) unless it is being performed on an aborted embryo or an organ which has been removed; and dividing cells are not usually obtained until the culture has run for at least 14 days, and often longer. This demands a proper tissue-culture laboratory, with facilities for all sorts of sterile procedures, and is thus much more expensive and laborious than lymphocyte culture; and the additional risk of infection or other accident to the cultures means a high failure rate. However the method also has its own advantages; cultures can be maintained for long periods for continued study, or cells may be deep-frozen (in a protective additive) to provide a cell bank which can be used years later as new techniques become available.

Cultures of this sort are also the only method available for the study of some tissues and material; studies on aborted embryos or on cells in amniotic fluid obtained by amniocentesis could hardly be performed by any other technique.

3. Direct Study of Cells Obtained by Marrow Biopsy

Apart from the testis, the bone marrow is the only certain source of *in vivo* dividing cells. Short term "cultures" can be carried out on bone marrow cells (this means in practice, exposure to colchine in a culture medium in order to accumulate metaphases which have already been initiated), and this should be a particularly appropriate method for the study of chromosome breakage (Chapter 13). One or two studies have been carried out on marrow cells after injection of colchicine *in vivo*, but doubts have been expressed as to the safety of the method and it has not been exploited (in any case it offers no advantage over the short term culture method).

Marrow is usually aspirated either from the sternum or the iliac crest by means of a wide bore needle. Although this is a fairly minor procedure it can be unpleasant for the subject, and is usually undertaken in hospital outpatient departments. It has mainly been used, therefore, on patients with blood disorders in whom a marrow biopsy is a diagnostic necessity, and is hardly justified as a purely experimental procedure.

4. Testicular Biopsy

This also is a procedure to which patients can hardly be subjected without good reason, but which affords excellent direct preparations of both meiotic and mitotic cells. Because of this difficulty in justifying the procedure, relatively few studies have been carried out in comparison with the other methods mentioned; but these have shown that testicular biopsy may often be a justifiable procedure in cases of infertility, and so it is to be expected that many more biopsies will be carried out in the future.

5. Dividing Cells in Epithelia

Methods have been devised for the study of dividing cells obtained from the roots of plucked hairs (Daker, 1970) but most workers have experienced difficulty in producing adequate spreading of chromosomes, and the method has not been widely used, though it would repay development since it can afford a direct way of obtaining dividing cells from a different type of cell from that usually studied.

III. MAKING CHROMOSOME PREPARATIONS FROM DIVIDING CELLS

A. Introduction

The problem of making chromosome preparations for the light microscope is in essence simple; it is the problem of converting a three-dimensional cell containing tightly-bunched chromosomes into a two-dimensional array of chromosomes in which each chromosome is separate from its neighbour.

The problem is easily overcome in the juicy cells of plants, by simply squashing the cells between a slide and a coverslip. But this method seldom works satisfactorily on animals because of the small size of the nucleus and the large number of chromosomes. Also it is hard to find adequate numbers of dividing cells in animal tissues; there are no sites comparable to the root-tip of a plant, where large numbers of dividing cells can always be found.

However the technical problems have been overcome, as outlined in Chapter 1, by the use of colchicine (or other antimitotic substances) to arrest cells at metaphase and so accumulate dividing cells; and by treating cells with hypotonic solutions in order to swell them up before squashing.

The essential stages in obtaining preparations of mammalian chromosomes are thus as follows:

(a) The cells are usually obtained in suspension; either because they have been cultured in suspension (as in blood cultures) or by suspending them in a physiological fluid. Fibroblast cultures, which grow in flat sheets, can be suspended by treatment with trypsin to separate them from each other (though this is not essential); marrow or testicular cells can be suspended by breaking up the tissue with a pipette in a physiological solution (or in some other way).

(b) The suspended cells are exposed to colchicine for a certain period.

(c) The cells are centrifuged, the colchicine solution removed by pipetting off the supernatant fluid, and a hypotonic solution is added and allowed to remain in contact with the cells for a certain, rather precise period.

(d) The hypotonic solution is removed by centrifugation and removal of supernatant fluid, and the cells are fixed by addition of a fixative which will not harden them too much for subsequent flattening to be achieved.

(e) The cells are flattened on to a slide, either by squashing or by an air drying technique (see p. 87).

(f) The preparation is stained, and, if necessary, mounted.

There are a number of possible variations on each of these stages; those which give the best results for a particular tissue can be ascertained by trial and error. Probably nearly every worker and laboratory uses slightly different methods, and there is a certain mystique involved. However the method of any experienced worker, in his own hands (though not always in the hands of others), will usually give excellent results. The following comments are offered from the author's personal experience.

B. Colchicine Pretreatment

The alkaloid colchicine (prepared from the autumn crocus) is a very potent substance, which has long been used for the empirical treatment of gout. Very low concentrations are adequate to inhibit mitosis. Its synthetic analogue, colcemid (CIBA), deacetylmethyl colchicine, is commonly used instead of the natural alkaloid. It is

30-40 times less toxic than colchicine (Schär *et al.*, 1954). A concentration of 4-6 parts per million will produce the desired effect. The optimum period of exposure of cells to this concentration depends on two factors; the time needed to collect sufficient cells at metaphase (for which the longer the exposure, the better) and the time needed to allow sufficient but not too great shortening of the chromosomes (which should not be too long). Optimum exposure time is therefore a compromise; too short exposure will produce a preparation with few dividing cells, in which the chromosomes may overlap because of their length; too long exposure will, in contrast, give many dividing cells, but the chromosomes in these will be so contracted that little can be seen of their morphology.

The optimum period of exposure varies with the tissue being examined, but for lymphocyte cultures 1½-2 hours is a good compromise. Some workers have however advocated much longer periods of exposure, of 4-6 hours or even longer. Fibroblast cultures require longer exposure to colchicine than lymphocyte cultures.

It is worth mentioning that it is quite possible to obtain satisfactory preparations without using colchicine at all. Dividing cells may be few, but may give exceptionally good chromosome morphology.

Other mitotic inhibitors, such as vinblastine sulphate, can be used in place of colchicine, and give comparable results.

C. Hypotonic Pretreatment

There are three variables in hypotonic pretreatment; the chemical nature of the hypotonic solution used, the degree of hypotonicity, and the length of exposure.

Many workers use sodium or potassium citrate as the hypotonic solution. Citrate was originally used by C. E. Ford *et al.* (1958) for work on bone marrow, in order to prevent clotting, and it is doubtful whether it has any other advantages over diluted physiological solutions such as Ringer's, Hank's or Eagle's balanced salt solutions, or even sodium chloride. Some workers prefer diluted human serum, claiming that it enhances chromosome morphology (Mellman, 1965). Potassium chloride has also been popular, and is believed to have some uncoiling or despiralizing effect. Unfortunately there have been few controlled studies of the use of different hypotonic solutions; most workers prefer to use a solution which has given good results in their hands.

Although it must be remembered that while the cells are undergoing hypotonic pretreatment they are still alive it is likely that

the uptake of water by the nucleus is largely a passive osmotic process, and there is little time for any specific metabolic effect of the substance used to be manifested; so that in the absence of controlled trials it is likely that given the right tonicity and time of exposure, any reasonably physiological substance will give satisfactory results.

It is usual to use a solution of about one-quarter physiological tonicity, for a period of 10-30 min. The time is rather critical for any given tissue; too short a time will lead to inadequate chromosome spreading, and too long a time to excessive breakage of cells, and also to loss of visible structure in the chromosomes (which appear bloated). It is necessary to determine the optimum time for any given tissue and solution by trial and error.

D. Fixation

Any fixative for chromosome work must produce minimal hardening. The only fixatives which have been widely used in practice are mixtures of methanol or ethanol and glacial acetic acid (acetic-alcohol, 1 : 3). Methanol is more popular than ethanol, and is believed to give slightly clearer preparations, but this has not been conclusively proved. It is always stated that the fixative should be freshly made and discarded after use (because of the formation of byproducts which interfere with good fixation), although once again it has not been possible to discover the origin or proof of this statement.

The period necessary for fixation depends on the amount of material to be fixed. Small amounts may be fixed in a minute or two, but a minimum of 20 min is usually recommended. Material which has been fixed may be kept in a refrigerator, in the fixative, for some weeks, with only slight deterioration, so clearly the time of fixation is not critical, provided it is long enough. Some workers prefer to fix at 4° C, but there is little evidence that this makes any difference.

It is also possible to fix in various concentrations of acetic acid in water, but this appears to offer no advantages over acetic-alcohol and has the serious disadvantage that cells stored in acetic acid deteriorate fairly rapidly, so that preparations require to be made immediately after fixation.

E. Chromosome Spreading

The cell membrane may be ruptured and the chromosomes spread either by squashing between slide and coverslip or by allowing the

cells in suspension to dry rapidly on a slide (when the cell is flattened by the surface tension as a meniscus passes rapidly over it).

Most of the early work on human chromosomes involved a squash technique adapted from plant cytogenetics (C. E. Ford and Hamerton, 1956). However airdrying techniques are probably more generally popular at present, being easier and quicker to employ, and demanding less skill. Squashing still has its advocates (notably in Scandinavia) and gives excellent results in skilled hands. In a typical squash technique the cells suspended in fixative are stained while in suspension, and a drop or two of suspension is squashed between slide and coverslip, usually with the thumb, but sometimes with a specially-designed press. Considerable pressure may be needed (often much of the body weight) but the process can be carried out gradually and observed at intervals in order to achieve the optimum degree of spreading. Excessive squashing (or movement of the coverslip on the slide) can result in smudging or disappearance of the chromosomes.

Squash preparations will keep for some weeks in a refrigerator if the coverslip is sealed to the slide with paraffin wax or Krönigs cement; or the coverslip can be removed after freezing, and permanent preparations made (Conger and Fairchild, 1953).

There are a number of different techniques for airdrying. The cells may be dried either from fixative or from an aqueous solution of acetic acid. If drying is from fixative, it is desirable to change the original fixative for fresh, and repeat this change twice or more, to remove excess water and debris. The most usual technique is to prepare slides by cooling in a refrigerator or in iced water, and then to drop a small quantity of the suspension of cells in fixative on to the cold, wet slide. The slide is then rapidly dried over the flame of a bunsen burner. When the alcoholic fixative meets the wet surface it spreads rapidly, giving wide dispersal of the cells. Another method of producing rapid drying is to ignite the fixative. These and comparable methods give good dispersion of cells on the slide, and on staining a faint cytoplasmic background to the chromosome spreads can be seen, which enables the observer to gauge whether a cell has been excessively or unevenly ruptured. The method is thus a good one when it is essential to obtain accurate counts on a large number of cells.

If spreading is done from an aqueous solution of acetic acid, the fixative is removed and two or three changes of acetic acid made. The commonest concentration is 45%, but higher concentrations may be used if greater dispersal of cell contents is desired. Drops of suspension are pipetted on to clean dry slides, which can be heated

on a hot plate up to 60°C to increase spreading. Drying can be expedited by touching the drops with a piece of blotting paper or tissue (the metaphase cells seem to stick to the glass of the slide, and are not thereby removed). This method gives concentric rings of cells on the slide. It gives excellent spreading and chromosome morphology but there are often many broken cells with a hypodiploid chromosome number so it is not recommended for chromosome counts. As the cells will deteriorate if kept in acetic acid, it is necessary to transfer them back to fixative if it is desired to keep them for any length of time.

F. Staining

The ideal method of staining chromosomes, because it is specific for their DNA content, is Feulgen's stain (Pearse, 1968), and this should be used if any quantitative work on DNA content is intended. However, for most ordinary purposes it is easier to use a number of good nuclear stains which give comparable, but deeper, staining. Perhaps the most commonly used is orcein, a naturally-occurring red dye which is usually made up in acetic acid, or in a mixture of lactic and acetic acids (lactic-acetic-orcein) at a concentration of 1-2%, which will stain most chromosomes adequately in a short time; but other stains such as Giemsa's stain, carmine, or even haematoxylin, can give quite satisfactory results.

In addition to these routine staining methods specific staining to show up banding patterns can be used; these involve either use of fluorochrome dyes, followed by ultraviolet microscopy, or Giemsa staining after special pretreatments (Chapter 2).

IV. THE EXAMINATION OF CHROMOSOME PREPARATIONS: KARYOTYPING

Karyotype analysis of satisfactory chromosome preparations may be made in one of three ways: by eye, by the use of photography, or by automatic methods involving the use of computers.

A. Karyotyping by Eye

Although visual karyotyping has not received much publicity, it is widely used, and by some of the largest laboratories working on cytogenetics. The use of the method has been described by Priest

(1969). It has the advantage that the chromosomes are actually examined on the slide, and each one can be carefully focused (whereas in photographs the quality of reproduction is often inferior to what can be seen by eye). The technique takes time to learn, and necessitates the making of drawings while learning, although these can be dispensed with when the eye is trained. It has the advantage that time is saved (results can be obtained almost immediately), and also money. The disadvantages are that there is no permanent record (other than a drawing) of the chromosomes, and that measurements cannot be made, so that identification of chromosomes by this criterion may be harder than in a photograph.

B. Karyotyping from Photographs

This is probably the most popular method of karyotyping. Two copies of a print will be needed (or more if there are overlapping chromosomes). The chromosomes are cut out, and laid in order according to one of the conventional methods (one is shown in Fig. 6), with the centromeres in each row on the same straight line, and the short arms upwards. Very precise workers, using very good preparations, claim that most if not all chromosomes can be allocated to their correct pairs on the basis of measurement and arm ratio. For the average preparation by the average worker, this is far from being the case, and the assignment of chromosomes No. 4-22 (except No. 16) and X to their correct pairs is very arbitrary. However with the new fluorescent and pretreatment-Giemsa-methods, nothing less should be aimed at than the goal of allocating each chromosome to its correct position.

C. Automatic Karyotyping

Automatic karyotyping has been under consideration for about 10 years, and much work has been done on the problems involved, which has been reviewed by Hilditch (1970) and Rutovitz *et al.* (1970). Some methods involve the scanning of photographs of metaphase plates, others are designed to work directly off a microscope. These methods involve preselection of the cells to be examined. Attempts have also been made to devise methods for the automatic selection of suitable cells for analysis.

These highly sophisticated methods are limited at present by the material available. If it were possible to produce with regularity chromosome spreads of superb quality, with no overlapping, and

with each chromosome displaying its correct relative length, automatic analysis would be readily achieved to an acceptable standard. But substandard preparations, with overlapping chromosomes, or chromosomes showing distortion of their arms, give rise to great technical problems of computer interpretation. Now that these are in process of solution, new staining methods, such as the fluorochrome staining methods, are introducing a new order of complexity into chromosome analysis. It therefore seems that it will be several years before computer-aided analysis of the human karyotype can begin to compete with human observation; the trained scientist is a far more sophisticated computer than the machine.

The day of automatic karyotype analysis will surely come; and when it does, the way will be open to population cytogenetic studies on a scale which cannot be attempted by present methods. But it does not seem worthwhile to consider it here in any detail, since the methods are only comprehensible to experts, and are not yet available for routine use; and by the time they do become available, anything which might be written here will be out of date.

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These references are not intended to be exhaustive (or even necessarily to give the best available description of each technique), but to give a good range of original papers describing workable techniques. To attempt to give a full bibliography of methodology would be beyond the scope of this book.

§ 5 §

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I. INTRODUCTION

Since this book is about chromosomes and how they work, it is necessary to give a brief account of the principles of molecular genetics insofar as these relate to chromosome structure and function. The following account is very basic, and is intended for those readers who are relatively unfamiliar with the subject. For a more adequate coverage of the subject it is suggested that recent textbooks such as those by Watson (1971) or Herskowitz (1967) be consulted.

II. NUCLEIC ACIDS

The nucleic acids were first isolated by Miescher (1871) from pus, a rich source of cell nuclei. Avery *et al.* (1944) were the first to show that purified nucleic acids were capable of producing genetic change, when they demonstrated that purified preparations could convert pneumococci forming "rough" colonies into ones which formed "smooth" colonies. Knowledge of the genetic significance of the nucleic acids has grown almost exponentially since that time, and the well-known climax of research on them was reached when Watson and Crick (1953) published their model of the structure of the deoxyribonucleic acid molecule (DNA); this model has stood the test of time and has served to explain how the genetic information contained in the chromosomes is utilized in the manufacture of the characteristic proteins of cells and organisms. It is now clear that DNA represents the genetic material of all living organisms, and contains the information necessary for their development, self-maintenance and reproduction.

A. Chemistry of Nucleic Acids

Nucleic acids are polymers of nucleotides. A nucleotide unit consists of a phosphate group, a pentose sugar and an organic base which is either a purine or a pyrimidine (both aromatic compounds).

The pentose of deoxyribonucleic acid (DNA) is 2′-deoxy-D-ribose. That of ribonucleic acid (RNA) is D-ribose.

The most common purines in DNA and RNA are adenine (A) and guanine (G). The most common pyrimidines in DNA are cytosine (C) and thymine (T), while in RNA they are cytosine and uracil (U). Thus RNA differs from DNA in its pentose sugar and in having uracil instead of thymine as its usual pyrimidine base. Other purines and pyrimidines can and do occur in nucleic acids, a fact often ignored in simple accounts of the subject.

A nucleoside is a combination of a pentose sugar and an organic base (purine or pyrimidine); when combined with a phosphate group it becomes a nucleotide.

The basic structure of the nucleic acid polymer is shown in Fig. 28. The backbone consists of alternate molecules of pentose and phosphate, while the organic bases are attached in line along one side of the pentose sugars. Any organic base can be attached to any pentose sugar in the chain, and this variable feature is the property which gives the nucleic acids their capacity to store information.

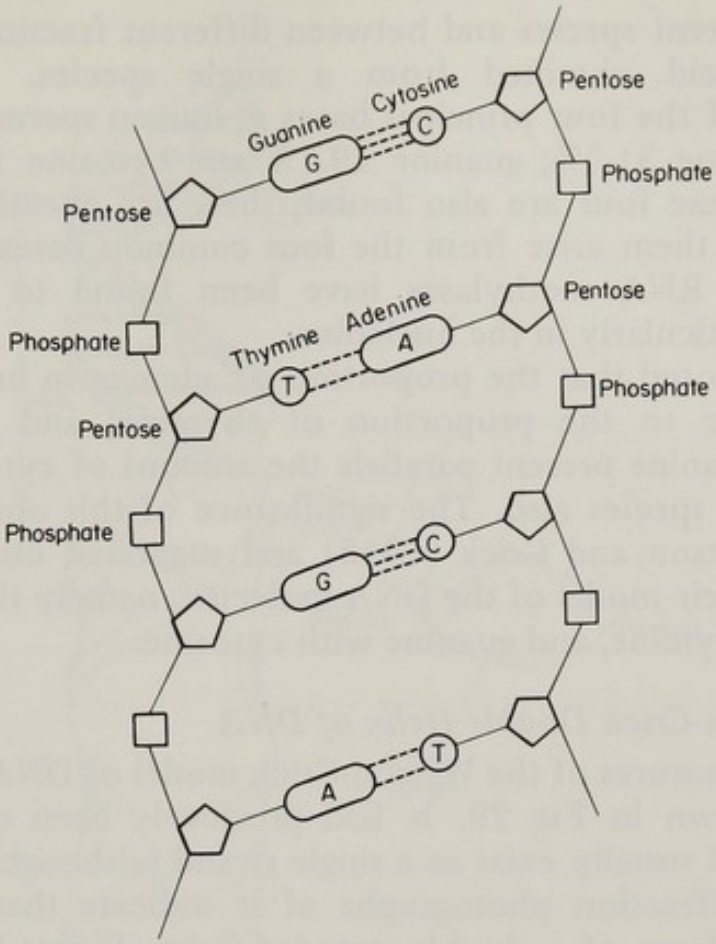

Fig. 28. Basic structure of DNA (diagrammatic). The helix is shown as a two-dimensional structure, with a backbone on either side consisting of alternate pentose and phosphate molecules. The two backbones have opposite polarities. To the medial side of each pentose is attached either a purine (A or G) or a pyrimidine (C or T). The two strands are held together by bonds (dotted lines) between either A and T or G and C. A = adenine, T = thymine, G = guanine, C = cytosine.

Another feature of the nucleic acid chain is that it is polarized; this means that the attachment of the phosphate groups to the pentose sugars is not symmetrical, so that the sequence of attachments will appear different if read in one direction along the chain to the way it will appear if read in the other direction.

Although there is no theoretical limit to the length a DNA molecule might attain, it seems that in practice the molecules have finite length. In the bacterium *Escherichia coli,* on which the pioneer research has been done, it seems that DNA segments of about 2.5×10^8 molecular weight are linked by proteins. Purified DNA from human spermatozoa contains about 0.1% of amino acid, which may be involved in such linkages.

Naturally occurring DNA and RNA vary considerably in the proportions of the different types of base which they contain, both

between different species and between different fractions (see later) of nucleic acid obtained from a single species. The relative proportions of the four principal bases in human sperm are: adenine 31.0%; thymine 31.5%; guanine 19.1% and cytosine 18.4%. Other bases than these four are also found; these are chemically related, and many of them arise from the four common bases by methylation; various RNA methylases have been found to be naturally occurring, particularly in the nucleolus.

It will be noted that the proportion of adenine in human DNA is closely similar to the proportion of thymine; and similarly the amount of guanine present parallels the amount of cytosine. This is true of other species also. The significance of this observation was noted by Watson and Crick (1953) and suggested one of the key features of their model of the DNA molecule, namely that adenine is paired with thymine, and guanine with cytosine.

1. The Watson-Crick Double Helix of DNA

The basic features of the Watson-Crick model of DNA proposed in 1953 are shown in Fig. 29. It had previously been observed that DNA does not usually exist as a single strand (although it can do so) and X-ray diffraction photographs of it indicate that it normally exists in the form of a double-stranded helix. Figure 28 shows the helix flattened out into a two-dimensional form. The two phosphate-pentose chains which compose it lie parallel to each other, with opposite polarities. The phosphate groups lie on the outside, and the two chains are joined to each other by chemical bonds between the organic bases, which lie centrally. A pyrimidine base on one strand is always paired with a purine base on the other. This feature allows the helix to have a constant diameter (if two pyrimidine bases, each containing only a single organic ring, were paired, they would not be long enough to bridge the gap between the two chains, while two purine bases, each containing a double ring, would be too long). If cytosine is paired with guanine, and thymine with adenine, the number of stabilizing bonds between each base pair is optimal.

In the three-dimensional configuration which exists in reality, the two phosphate-pentose backbones of the two strands are coiled round each other, rather like the strands of a rope, and make a complete revolution every 34 Å. There are ten nucleotides in each complete turn, so that each nucleotide takes up 3.4 Å of the strand.

Another significant feature of the Watson-Crick molecule is shown diagrammatically in Fig. 29. The cross-sectional configuration of the

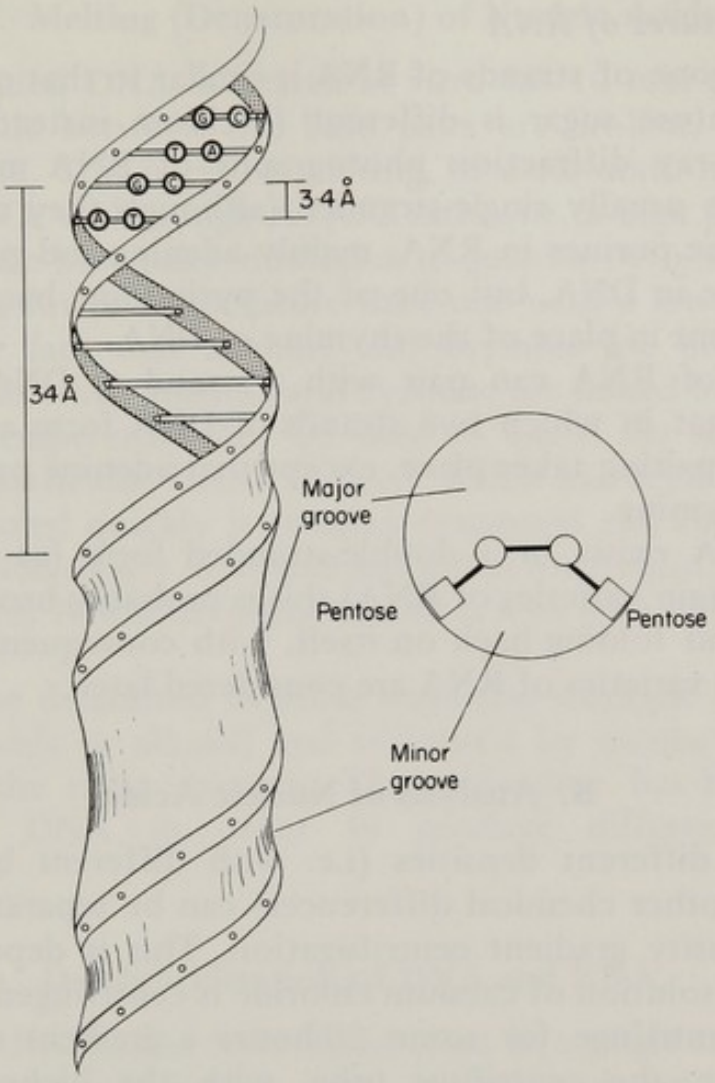

Fig. 29. The DNA double helix (after Watson and Crick, 1953). The lower half of the lefthand figure has been filled in to give an impression of the major and minor grooves. The righthand figure gives a cross-section through one of the central nucleotide pairs, to show how the angulation between them and the pentoses gives rise to major and minor grooves.

molecule is such that there are two helical grooves running around the sides of the helix, the "major" and "minor" grooves. These are of importance in the relationship of the molecule to other molecules, in particular, histone proteins, which are thought to "intercalate" into the grooves to form nucleoprotein, the complex of nucleic acid and protein which constitutes the chromatin of the cell nucleus. Histones and protamines (another form of protein which is associated with nucleic acids, notably in the sperm head) are probably attached to the DNA by electrostatic bonding between their amino groups and the phosphate groups of the DNA. Apart from naturally occurring substances which intercalate into the major and minor grooves, it is also thought that certain stains (such as the fluorochromes and methylene blue) may intercalate into these grooves.

2. The Structures of RNA

The backbone of strands of RNA is similar to that of DNA except that the pentose sugar is different (D-ribose instead of 2′-deoxy-D-ribose). X-ray diffraction photographs of RNA molecules show that they are usually single-stranded (although they can be double-stranded). The purines in RNA, mainly adenine and guanine, are the same as those in DNA, but one of the pyrimidine bases is different; uracil is present in place of the thymine of DNA.

A strand of RNA can pair with a strand of DNA in the same manner as that in which two strands of DNA form a double helix. Similar base pairing takes place, except that adenine pairs with uracil instead of thymine.

When RNA exists in a double-stranded form (as appears to be usual for certain varieties of RNA) this is probably brought about by a single strand folding back on itself, with consequent base-pairing. The different varieties of RNA are considered later.

B. Analysis of Nucleic Acids

DNAs of different densities (i.e. with different base-pair compositions or other chemical differences) can be separated from each other by density gradient centrifugation. This is dependent on the fact that if a solution of caesium chloride is centrifuged at high speed in an ultracentrifuge for some 20 hours a gradient of densities is established in the centrifuge tube, with the highest density of solution at the bottom of the tube. If DNA is centrifuged in such a tube it takes up a position corresponding to its own density. Thus DNA of higher or lower density can be distinguished by its position in the tube (which can be detected by making use of a physical property of DNA, its capacity to absorb ultraviolet light of 2600 Å wavelength). Using this technique it can be shown that the main bulk of human DNA fractionates together, but that about 2.5% of it forms a very distinct fraction of different density (Corneo *et al.*, 1970). This separate fraction of DNA has been called "satellite DNA", and can itself be separated into two separate components. A further 15% of the total DNA has a slightly different density from the remainder, and has been called "homogeneous main band DNA". Satellite DNA has a different proportion of A-T and G-C base pairs from the bulk of DNA (which accounts for its different density) and this is thought to be due to the presence in it of many highly repetitive sequences of bases ("redundant DNA").

C. Melting (Denaturation) of Nucleic Acids

If two-stranded DNA is heated *in vitro* for 10 min at 100°C the hydrogen bonds between the base pairs are broken, and the two strands separate. This is called melting, or denaturation. It has been found that DNA with a high AT/GC ratio (i.e. a high proportion of adenine-thymine base pairs in relation to guanine-cytosine base pairs) denatures at a lower temperature than one with a lower ratio. This relates to the fact that adenine and thymine are linked by two hydrogen bonds while guanine and cytosine are linked by three. Thus the melting temperature can be used to assess the base-pair ratio present in a particular DNA. If DNA which has been denatured in this way is cooled quickly it remains denatured and single-stranded, but if it is cooled slowly, base pairing occurs once again, and it is said to be renatured. A similar phenomenon is seen with double-stranded RNA.

DNA can be denatured in other ways (for example by treatment with strong acids or alkalis) and renatured by incubation in saline solutions of the right strength. This procedure has been used on chromosomal DNA in order to produce differential staining (Chapter 2).

D. Hybridization of DNA and RNA

DNA and RNA can be hybridized (that is, a single strand of DNA can be made to base-pair with a single strand of RNA) if single-stranded RNA is filtered through agar containing denatured DNA from the same organism, indicating that at least some of the RNA in that organism is complementary to its own DNA. It is also possible to make DNA-DNA hybrids between different organisms if the organisms are sufficiently closely related, and this technique can be used to determine the similarity (or difference) of base-pair sequence in the DNA and/or RNA from the same or different organisms.

III. TRANSCRIPTION OF NUCLEIC ACIDS

When a double-stranded helix of DNA replicates (i.e. forms a replica of itself) *in vivo*, two new double strands are produced. Each of these new strands contains one of the original parental strands and

one newly-formed strand (Fig. 30). This form of replication is referred to as semiconservative, and has been proven at the molecular level by experiments on bacterial DNA. If bacteria are allowed to grow and multiply in a medium containing the heavy isotope of nitrogen ^{15}N instead of the usual ^{14}N the newly-formed DNA will take the isotope up into its bases and will be "heavy DNA". This can be separated from ordinary DNA by density gradient centrifugation.

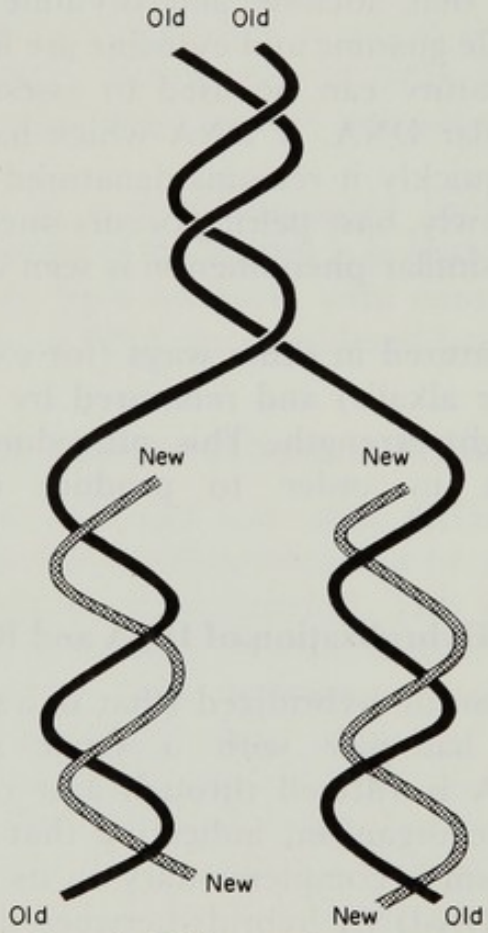

Fig. 30. Diagram showing how the DNA strands separate at replication, each old strand acting as the template for a new one (after Watson, 1971).

If the bacteria are grown in heavy nitrogen for several generations so that virtually all their DNA is heavy, and then allowed to grow for a single generation in a medium containing ordinary (light) nitrogen it is found that their DNA is exactly intermediate between heavy and light DNA. If they are allowed to grow for a further generation, the isolated DNA is of two sorts; half is intermediate as before and half is light.

Chromosomes have been shown to replicate semiconservatively (Taylor, 1965) and this is without doubt due to the semiconservative replication of their contained DNA.

When a double-stranded strand of DNA forms two new double strands by this process of semiconservative replication, it is clear that the base pairs in the new double strands will be identical to those in the original. This process whereby the base sequence in a parental strand is transferred to a new strand is called *transcription.*

In vivo double-stranded DNA may be transcribed to new double-stranded DNA (or to single-stranded DNA) by the enzyme DNA polymerase. Double-stranded DNA can also be transcribed to single-stranded RNA by DNA-dependent RNA polymerase. In this instance, of course, the sequence of bases in the RNA so formed will be complementary to those in the parent strand of DNA and will include uracil instead of thymine.

Whilst *in vivo* transcription is normally from DNA to DNA or DNA to RNA, there is now evidence that transcription from RNA to DNA can occur. The Rous sarcoma virus of fowls consists of single-stranded RNA. When it has infected chick embryos, DNA is found in their cells which can be hybridized with the virus; such DNA is not found before infection, indicating that transcription of viral RNA has probably taken place; the enzyme involved in this has been called reverse transcriptase.

What has been said so far may be summarized by saying that DNA has a structure such that an almost infinite variety of information may be contained in it; this information resides in the different sequence of the four principal bases (and to a lesser extent of other bases also) along the length of the molecule. This information can be transcribed indefinitely to other DNA which is formed semiconservatively from the original strand, and is thus a precise copy of it; and it can also be transcribed to single-stranded RNA and utilized, as will be described in the next section, as the basic information necessary for protein synthesis.

IV. TRANSLATION OF NUCLEIC ACIDS: THE GENETIC CODE

Transcription of nucleic acids only involves a single language, whose alphabet consists of the four letters comprised by the four principal purine or pyrimidine bases of the DNA molecule. Protein synthesis however involves the manipulation of 20 amino acids into the correct sequences. Thus a *translation* of the language of DNA into the language of proteins (or their polypeptide subunits) becomes necessary. Three varieties of RNA are involved in this process.

These three types of RNA are: (a) Messenger RNA (mRNA); (b) Ribosomal RNA (rRNA); (c) Transfer RNA (tRNA), which is also called soluble RNA (sRNA).

Messenger RNA is single-stranded RNA which is transcribed from the DNA in the chromosomes in the nucleus, and then passes to the cytoplasm where protein synthesis takes place.

Ribosomal RNA is present in the ribosomes, small, discrete cytoplasmic bodies which are readily visible to the electron microscope. Ribosomes can be characterized by their rate of sedimentation, measured in sedimentation units (s). Ribosomal RNA is single-stranded and has rather high molecular weight. It is probably mainly transcribed on the nucleolar-organizing regions of the chromosomes, particularly on the satellite-associated secondary constrictions on chromosomes 13-15 and 21-22. In rats and mice rRNA is complementary to about 0.3% of the total DNA. It is thought that the DNA in the nucleolar-organizing regions of the chromosomes may consist of long chains of repeating genes ("ribosomal cistrons").

Transfer RNA is another transcript of DNA which is of low molecular weight (about 26,000). It is thought to consist of a single rather short single-stranded RNA molecule which base-pairs with itself by folding back on itself to form a configuration rather like a clover-leaf. There are some scores of different tRNAs, each of which is different in its sequence of bases, but each of which comprises some 20 different groups of bases ("codons"); each of these groups is thought to correspond to a different amino acid. tRNA is soluble in molar NaCl (which rRNA is not) which is why it is sometimes called soluble RNA (sRNA).

Translation of the message carried in the mRNA into the correct sequence of amino acids for the manufacture of a specific protein is thought to proceed as follows:

Amino acids in the cytoplasm become attached to their own specific molecules of tRNA. When the mRNA reaches the cytoplasm, the ribosomes thread themselves on to it one after another (so that often several are attached simultaneously) and they pass along the thread of mRNA from one end to the other, where they become free again. As they pass along, they cause the molecules of tRNA, carrying their individual amino acids, to be attached in turn to their specific codons. As each pair of such molecules of tRNA with attached amino acid are next to each other, the amino acids become linked, and so a chain is built up as the ribosomes move along (Fig. 31). The polypeptide chain so formed will be determined by the length and base sequence of the original strand of mRNA.

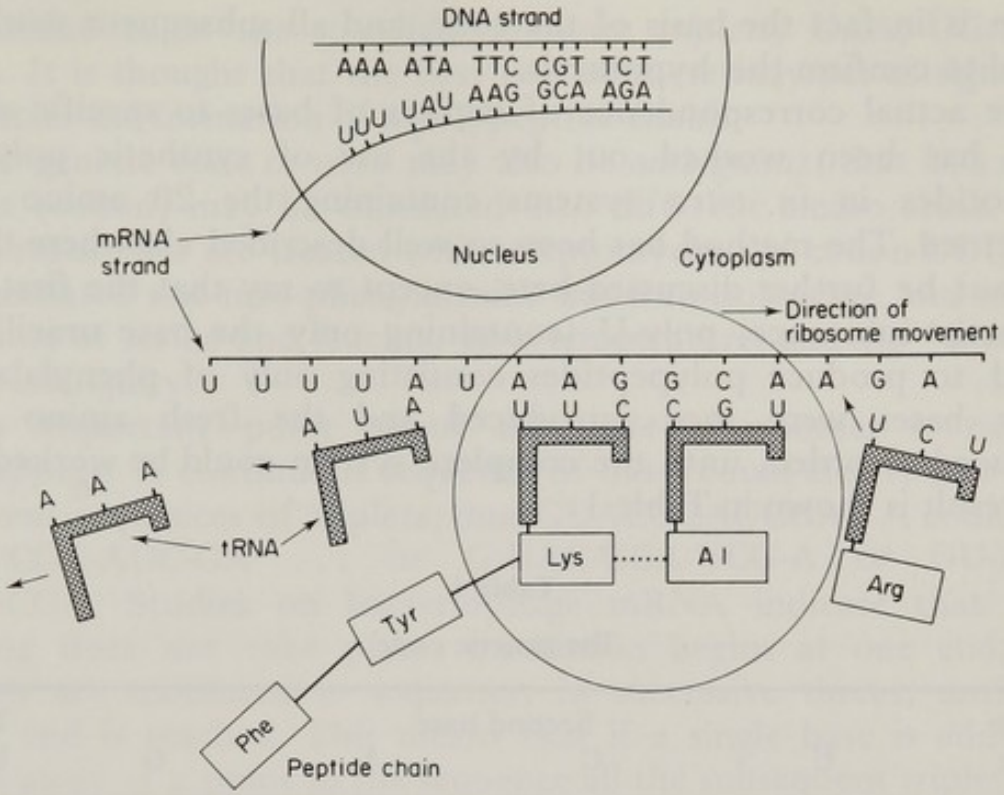

Fig. 31. Transcription of DNA to mRNA (above), and translation of information on mRNA to form a peptide chain (below). The lower circle represents a ribosome moving along the strand of mRNA. Phe = phenylalanine, Tyr = tyrosine, Lys = lysine, Al = alanine, Arg = arginine.

A long mRNA molecule with a whole sequence of ribosomes attached to it is called a polyribosome, and these structures are readily visible to a high-resolution electron microscope.

mRNA has a definite and limited life, after which it is degraded. Studies on bacteria in which transcription of DNA to RNA has been blocked with actinomycin D indicate that in this system it has a life of about 2 min, during which a molecule may be translated some 10-20 times. It is not known how it is then degraded, but ribonuclease (RNase) is thought to be involved.

1. The "Genetic Code"

Clearly a single base on mRNA cannot correspond to a single amino acid, since there are 20 of the latter, and only four bases. Certain combinations of bases must correspond to each amino acid. Such combinations, each corresponding to a single amino acid, are called *codons.* A codon consisting of two bases only could only give 16 different combinations—again not enough; but a codon of three bases could give 64 possible combinations, which would be more than adequate. Crick *et al.* (1961) hypothesized that a three-base

codon is in fact the basis of the code, and all subsequent work has served to confirm this hypothesis.

The actual correspondence of triplets of bases to specific amino acids has been worked out by the use of synthetic polyribonucleotides in *in vitro* systems containing the 20 amino acids concerned. The method has been so well described elsewhere that it will not be further discussed here, except to say that the first such synthetic substance, poly-U (containing only the base uracil) was found to produce polypeptides consisting only of phenylalanine. Other bases were then introduced and the fresh amino acids produced recorded, until the complete system could be worked out. The result is shown in Table 1.

Table 1

The genetic code

First base	Second base U	C	A	G	Third base
U	Phenylalanine	Serine	Tyrosine	Cysteine	U
	Phenylalanine	Serine	Tyrosine	Cysteine	C
	Leucine	Serine	nonsense	?	A
	Leucine	Serine	nonsense	Tryptophan	G
C	Leucine	Proline	Histidine	Arginine	U
	Leucine	Proline	Histidine	Arginine	C
	Leucine	Proline	Glutamine	Arginine	A
	Leucine	Proline	Glutamine	Arginine	G
A	Isoleucine	Threonine	Asparagine	Serine	U
	Isoleucine	Threonine	Asparagine	Serine	C
	Isoleucine	Threonine	Lysine	Arginine	A
	Methionine	Threonine	Lysine	Arginine	G
G	Valine	Alanine	Aspartic acid	Glycine	U
	Valine	Alanine	Aspartic acid	Glycine	C
	Valine	Alanine	Glutamic acid	Glycine	A
	Valine	Alanine	Glutamic acid	Glycine	G

It will be noted that some amino acids (all in fact except methionine and tryptophan) are coded for by more than one triplet of bases. This is called degeneracy of the code. The degeneracy relates most to the third base of the triplet, although the first is also involved to a lesser extent. Conversely (and as one might expect in view of the fact that there are so many more possible triplets than there are amino acids) there are some triplets which do not code for

any amino acids—the so-called *nonsense triplets*, UAA, UGA and UAG. It is thought that the first two of these may act as signals to terminate the formation of a polypeptide chain.

The genetic code *in vitro* may also be *ambiguous*; that is, a single triplet (codon) may be translated into different amino acids. Thus when ribosomes are treated with streptomycin the codon UUU may be translated not into phenylalanine but into isoleucine, and smaller amounts of serine and leucine. Low temperatures and Mg^{2+} ions also cause ambiguity.

An important point about the code is whether it can be *overlapping*. A continuous sequence of bases could clearly give three different sequences of triplets; thus GUACGCAUCGA . . . could give GUA-CGC-AUC-GA . . . or G-UAC-GCA-UCG-A or GU-ACG-CAU-CGA. Studies on bacteriophage mRNA indicate that overlapping does *not* take place; translation begins at one end, and triplets are translated in sequence, in successive threes, until the other end is reached. This means that if a single base is added or taken away at a point in the sequence all the subsequent triplets will be read out of phase, and will code differently. Triplets altered in this way may code in three different ways; as *sense* codons (coding the same as before); as missense codons (coding for a different amino acid) or as *nonsense* codons—coding for no amino acid, and possibly terminating the polypeptide at that point.

In vivo the code is even more degenerate than it is *in vitro*. This is because there may be more than one RNA for a given amino acid. Thus it has been shown that the tRNA for leucine in *E. coli* can be of three different types with different coding properties. This may relate to the fact that so many amino acids are coded for by more than one triplet. The *in vivo* code may also be ambiguous; the wrong amino acid may get linked to a particular tRNA, or the wrong *anticodon* may pair with a particular mRNA triplet.

Most of the fundamental work on the genetic code has been performed on very lowly organisms—bacteria, viruses and bacteriophage. It is very pertinent to ask whether it is in fact universal, and whether the findings on these simple organisms can equally well be applied to the enormously more complicated organization of a mammal such as man. All experiments so far on mixed *in vitro* systems incorporating material from lower and higher organisms indicate that the basic code *is* universal, and that polypeptides are synthesized in a similar manner in the lowest and the highest forms of life. Obviously differences exist in the way in which the operation of the code is regulated; it is probably in the greater complexity of

this that the greater complexity of higher organisms is founded. This subject will be discussed in the next section.

V. THE CONTROL OF RNA AND PROTEIN SYNTHESIS

Our knowledge of the mechanisms which control the information contained in the DNA, and which determine which parts of the genome (the total amount of an individual's genetic material) is transcribed at any given moment is still extremely rudimentary, particularly in higher organisms. Such mechanisms must be of great importance in cell differentiation, and it is very clear that in the adult, the cells in different organs produce a very different set of proteins. In any given cell it is to be presumed that only a small proportion of the genome is active; perhaps in highly differentiated cells large parts of it have had their activity permanently suppressed. But little fundamental work has yet been done on this subject, because of its complexity. The most fruitful research so far has been done on the regulation of gene action in bacteria, and the next section will deal concisely with this work.

The pioneer concept in this field is that of Jacob and Monod (1961). They suggested that there exist *operator* genes which do not produce, as does the rest of the genome, any unique, detectable product, but whose action is to control the action of other genes. These genes have also been called *functional genes* (as opposed to *structural genes,* which specify particular chemical structures). The evidence for the existence of such genes is as follows:

Regions have been found in the genome of *E. coli*, such as the *lac* region. This region produces a small group of enzymes (let us call the genes concerned *a*+, *b*+ and *c*+ (the + indicates that they are functional). However it is found that these enzymes are not produced except in the presence of lactose. This is referred to as an *inducer* substance. There is evidence that lactose acts by inactivating a *repressor* substance, which is probably a protein, and therefore produced by another gene, *r*+, which is called a *regulator* gene. In the absence of lactose, this gene produces its repressor substance, and the genes *a, b* and *c* are unable to function.

However there is evidence that the system is more complicated still. *lac* regions are found in certain bacteria which can produce the *a, b* and *c* enzymes even in the absence of lactose (this is referred to as *constitutive* production, as opposed to the normal *inductive* production in the presence of lactose). Yet when these regions are

inserted into other bacteria which lack a regulator gene *r*+ they can be shown to produce the repressor substance which will suppress enzyme production in that bacterium. Therefore they must be *r*+. The simplest explanation of this state of affairs is that the repressor substance produced by the regulator gene *r*+ acts on another gene, the *operator* gene, *o*+, which controls the action of the series of enzyme-producing genes *a*, *b* and *c*. If this operator gene is mutated to an inactive operator gene *o*−, then the genes *a*, *b* and *c* can function constitutively even in the presence of the repressor substance. The state of affairs is summarized in Fig. 32. A group of genes controlled by a single operator gene is called an *operon.* There is good evidence that the genes in such systems have linked functions; that is, they may code for enzymes which are needed in successive stages of a particular metabolic pathway; for example, in *Salmonella* a gene cluster has been demonstrated which produces ten enzymes active in the synthesis of histidine—the histidine operon.

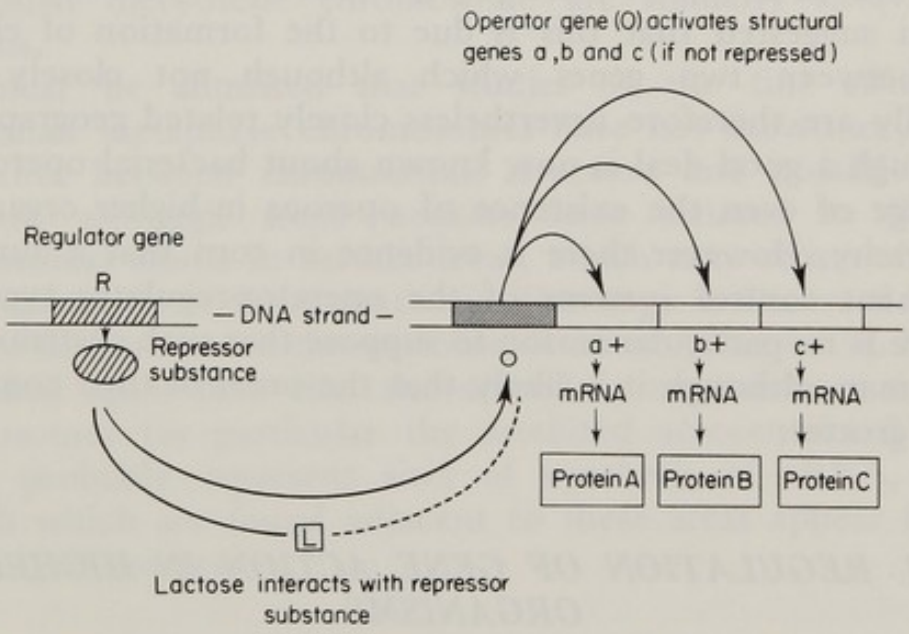

Fig. 32. The action of operator (O) and regulator (R) genes. L = lactose (inducer substance). For explanation see text.

Regulator genes can control operons in two ways; they may, as in the example given of the *lac* operon, produce an *active* repressor substance, which is inactivated by a metabolite; or they may act in the reverse manner and produce an *inactive* repressor which is activated by a metabolite (the histidine operon of *Salmonellae* is thought to be of this sort). Operons with an active repressor will most commonly be inactive themselves, while operons with an inactive repressor will usually be active.

An operon may be considered as a unit of gene function. There is evidence that the structural genes in an operon are transcribed on to a single strand of mRNA. It is not known what causes the termination of translation of the mRNA—possibly a nonsense triplet, or a doubling-back of the end of the single strand of RNA so that it base-pairs with itself.

There is further evidence from bacterial operons that operator genes in different operons may have rather similar nucleotide sequences; and this may also be true of regulator genes.

A final important point concerning operon systems in bacteria is that there is good evidence that chromosomal folding may bring together operons which are not closely genetically linked but which are functionally related. Thus in *E. coli* it appears that eight genes concerned in arginine synthesis, and which are all controlled by the same regulator gene, reside in three separate operons. After irradiation with UV light a significant number of bacteria are found which have mutations in two of these genes, which are not closely linked. It has been suggested that this is due to the formation of chemical bonds between two genes which although not closely linked genetically are therefore nevertheless closely related geographically.

Although a good deal is now known about bacterial operons, our knowledge of even the existence of operons in higher organisms is very sketchy. However there is evidence in corn that a number of two-element control systems of the operator-regulator type exist; and there is no particular reason to suppose that such systems do not exist in man, although it is likely that the order of their complexity is vastly greater.

VI. REGULATION OF GENE ACTION IN HIGHER ORGANISMS

Although we know little in higher organisms about single-gene interactions of the sort which have just been described, a certain amount is known on a grosser scale, about sites of synthesis and factors which may affect them, and some of these will now be considered.

A. Sites of Transcription

Some sites of transcription can be recognized cytologically in specialized chromosomes of higher organisms. In the polytene

chromosomes of Diptera (*q.v.*) certain areas can be seen to form "puffs", areas in which the chromosome seems partly to unravel itself. Such puffs have been proved to be sites of transcription. Similarly, in the *lampbrush chromosomes* of amphibian oocytes (*q.v.*) the lateral loops have also been shown to be sites of RNA formation. Autoradiographic studies using tritiated uridine show that this is incorporated into newly-formed RNA in these loops, starting at the thin end and proceeding to the thick end, over a period of some 10 days. The loops contain a strand of DNA, and if treated with actinomycin D (which inhibits RNA synthesis) they appear to disappear.

Since the lampbrush chromosome structure has been seen in mammalian oocytes, and indeed may represent a basic model of chromosome structure, sites of synthesis in the chromosomes of some higher organisms can be deduced by analogy. Thus Baker and Franchi (1967) suggest that the lampbrush-like loops of the mammalian dictyotene chromosome are similarly sites of RNA synthesis.

It must be admitted that studies of the fine structure of mammalian metaphase chromosomes have not shown any obvious correlation between chromosome structure and possible sites of synthesis, although some workers have claimed to find finer chromosomal fibrils in certain areas, which may represent sites of DNA replication or RNA synthesis. This topic is discussed in greater detail in the section on chromosome structure (Chapter 6). It may be mentioned again here that the secondary constrictions on certain chromosomes (in particular the satellited acrocentrics 13-15 and 21-22) probably represent sites of synthesis of rRNA, and the nucleoli which are found adjacent to these areas appear like great masses of ribosomes.

B. Genetic Regulation of Development

There are considerable differences in the proportions and types of RNA found in cells in different organs in the body, and this must, as has been said before, relate to differential action of the total DNA in RNA synthesis in differentiated cells. However almost nothing is known of the regulation of this. A little work has been done on Anurans (frogs and toads) on the sorts of RNA formed during the earliest stages of development. It has been found that during oogenesis the RNA formed is mainly rRNA, which is conserved against future activity in protein synthesis. After fertilization, the

mature oocyte produces some RNA during the early stages of cleavage. Only at the time of gastrulation does the formation of mRNA (and further rRNA, leading to the production of nucleoli) commence. The regulatory mechanism for all this is unknown.

C. Differential Action of Proteins on Transcription and Translation

1. Transcription

The way in which proteins, particularly histones, are bound to DNA almost certainly affects transcription in higher organisms. Information is rapidly accumulating about the chemical nature of the hitherto rather mysterious histones (Phillips, 1971). Five major fractions can be discovered from preparations of nuclear histones:

(a) A very lysine-rich fraction with molecular weight about 21,000.
(b) Two moderately lysine-rich fractions with molecular weights of about 14,000.
(c) Two arginine-rich fractions with molecular weights of about 14,000 and 11,000.

The last of these fractions shows remarkable stability of structure in higher organisms. Only a few amino acid differences are found in fractions of it derived from peas and from calves.

The five fractions are found in all chromatin and at all times (with the solitary exception of the DNA of the mature sperm head of many animals, where histones are replaced by protamines).

All the fractions show a curious asymmetry in their amino acid sequences. In the lysine-rich fraction basic groups tend to be found in the carboxy-terminal half, and hydrophobic groups in the amino-terminal half; in the moderately lysine-rich and arginine-rich fractions the converse is true. The basic halves of the molecules are rich in antihelical amino acids (glycine or proline).

The histones are thought to associate with DNA through interaction between their amino groups and the phosphate groups of the DNA. The arginine-rich histones may bind to the major spiral of the DNA by their non-helical basic ends. They are thought to play an important part in the complex folding of DNA in higher organisms, possibly by the formation of hydrogen bonds between pairs of histones.

Other proteins beside histones are linked to DNA at various sites, and it has been suggested that they may be present in orderly

functional arrays (DuPraw, 1968), made up of such enzymes as RNA polymerase, NAD synthetase, nucleoside triphosphatase, histone acetylases, phosphoproteins, perhaps DNA polymerase and enzymes of nuclear oxidative phosphorylation. In addition it has been suggested that a type of RNA which is resistant to RNase (chromosomal RNA) may play a part in binding histones to chromosomes.

Different histones have different effects on the denaturation of DNA *in vitro.* Lysine-rich histones, when combined with DNA, raise the temperature at which half the DNA present is denatured (the half-melting temperature) by 10° C. Arginine-rich histones (which are poor in lysine) have almost no such effect; nor do protamines, which are also arginine-rich.

Arginine-rich histones can inhibit RNA synthesis in isolated nuclei from calf thymus; lysine-rich histones also have some effect, but it is much less.

It has been shown that cells which are actively growing and synthesizing protein have a much greater proportion of their DNA uncomplexed with protein than do less active cells. So there are strong indications that histones play a part in inhibiting RNA synthesis by complexing with DNA in various places. Whether this is largely a mechanical effect brought about by such complexing (in which there may be intercalation into the major and minor grooves of the DNA) or whether the effect is more complex, is not yet known. However there is some evidence that acetylation of histones may play some part in the process. *In vitro* synthesis of RNA seems to be greater when the proportion of acetylated histones rises.

2. Translation

There is evidence to suggest that proteins are capable of inhibiting translation by combining with ribosomes. Mammalian interphase ribosomes are translationally active *in vitro.* Metaphase ribosomes are however fairly inactive. Their activity can be restored by treatment with trypsin, which presumably removes the protein.

D. Position Effects and Gene Action

There are a number of examples in *Drosophila* of genes whose functioning is affected by the nature of the neighbouring genes, although their structure is unaffected. This may sometimes be due to changes in coiling, or heterochromatinization. If euchromatic segments of *Drosophila* chromosomes come (through chromosomal

alterations) to lie nearer than usual to heterochromatic segments, they may become heterochromatic themselves; conversely heterochromatic segments may become euchromatic if positioned near euchromatic regions of the chromosomes. This mechanism has not yet been demonstrated in mammals.

E. Dosage Compensation

This term is given to the phenomenon seen when one sex has two active sets of particular genes, and the other only one (as for the genes of the X chromosome in man and most mammals); in most cells one set of alleles is completely suppressed (or largely suppressed) in the homogametic sex; in man by the inactivation, in any given cell, of one of the two X chromosomes. This will be discussed in greater detail elsewhere (Chapter 7) but is mentioned here as an obvious example of a regulatory mechanism.

F. Hormones and Gene Activity

Hormones which alter the activity of cells distant from their origin probably frequently act by regulating gene action. Certainly they can stimulate mRNA and protein synthesis in certain cells—for example the action of oestrogens on the uterine endometrium. It is known that some hormones may alter the melting properties of DNA. One may speculate that other hormones might act on operator or repressor genes, or even act as repressors or derepressors themselves.

G. Production of Antibodies

When leucocytes (lymphocytes or plasma cells) are exposed to an antigen, they begin to synthesize ribosomes and mRNA at a high rate, and then produce large quantities of the corresponding antibody, i.e. one specific protein. This is usually associated also with repeated cell divisions. The antibody is a unique substance, and here a more complicated mechanism than a mere transcription of a portion of the genome seems necessary to explain matters.

H. RNA Synthesis and Memory

There is evidence in rats that RNA synthesis takes place in neurons involved in the learning process. Cortical neurons synthesize RNA which early in the learning process appears to be rich in adenine and

uracil; later the RNA has a base ratio similar to rRNA. It has been suggested that these two stages may relate to short- and long-term memory.

VII. CONTROL OF DNA REPLICATION: THE REPLICON HYPOTHESIS

A. Replication in Simple Organisms (Prokaryotes)

Most detailed knowledge of molecular biology comes from experimental work on very lowly organisms, such as bacteriophage, viruses and bacteria. These and the blue-green algae lack nuclear envelopes, mitotic spindles, and other features of higher organisms and have been called prokaryotes (or "prokaryotic"), whereas more complex organisms which possess these structures have been called eukaryotes or eukaryotic, which means truly nucleated (Dougherty, 1957). Semiconservative replication of DNA has been proved both for prokaryotes and eukaryotes; but the prokaryotes have so much less DNA in them that it can be considered as replicating as a single unit, while the eukaryotes have so much DNA that it must form many replicating units. It has often been pointed out that the total amount of DNA in a mammalian nucleus must represent about 1.5-2 m of Watson-Crick molecule. If this were to replicate as one unit it could not possibly unwind its two daughter strands in the time in which cells are known to replicate. This does not necessarily exclude the hypothesis that all the DNA in a eukaryote nucleus might belong to a single molecule; it would be possible for such a molecule to be broken at various points at replication and later reunited by appropriate enzymes (ligases).

There are two further distinctive differences between the DNA of prokaryotes and eukaryotes. Prokaryote DNA is naked (i.e. it is not complexed with protein), and the gene sequences it contains are almost entirely unique; while eukaryote DNA is complexed with protein as previously described, and usually shows a considerable degree of redundancy; some sequences of genes (or more precisely, of cistrons) are repeated many times, sometimes many thousands of times. DNA containing such sequences is referred to as redundant.

Jacob *et al.* (1963) have put forward a *replicon hypothesis* for the regulation of DNA replication, based on experimental work on bacteria. This is analogous to the operon hypothesis of gene action in that it presupposes that replication takes place in units, called

replicons, which possess a replication locus comparable to the operator locus in operons; and also presupposes a replicator substance. In the prokaryote *Escherichia coli* the bacterial "chromosome" consists of a DNA helix about 1.1-1.4 mm long, and this appears to replicate as one continuous unit or replicon, at the rate of 20-30 μ/min (Cairns, 1963). DNA hybridization studies indicate that the DNA sequences are unique; there is no repetition, except of the DNA coding for the ribosomes, which may exist in 5-6 copies. The simple structure of this unit makes the term "chromosome" rather inappopriate and a better word which has been devised for it is "genophore". A genophore is defined as any structure which is associated with genetic linkage groups. Prokaryotes possess a single genophore, which acts as a single replicon. Eukaryotes have, by definition, two or more (and usually many) genophores, and an even larger number of replicons.

B. Replication in Eukaryotes

Most mammals, including man, have about the same quantity of DNA present in the nucleus. In man this has been estimated as being equivalent to 160-170 cm of Watson-Crick molecule (DuPraw, 1968; Ris and Kubai, 1970). This is packed into about 220 μ of chromosome, and hence must be very highly folded. Ris and Kubai (1970) offer as an analogous concept the problem of packing a pipe-cleaner 240 km long into a cylinder 8 m long and 1 m diam.

Rudkin (1967) has estimated the DNA content of individual human chromosomes, and finds it to relate approximately to chromosome size; he estimates that chromosome No. 1 contains about 7.3 cm of Watson-Crick molecule; and an average chromosome contains about 3 cm.

Eukaryote DNA replicates much slower than prokaryote DNA. This probably relates particularly to the presence of a protein covering, with the more complex chemistry involved (and also the necessity for the protein itself to be replicated). Taylor (1968) estimated from autoradiographic experiments that the rate of replication of DNA in the Chinese hamster is about 2 μ/min, and Cairns (1966) using similar techniques on Hela cells (a strain of malignant human cells kept in continuous culture for many years) estimated that their DNA replicates at about 0.5 μ/min. Simple arithmetic indicates that there must be at least 5000 separate sites of replication in the Chinese hamster (which will be 20-100 μ apart) to allow replication of all the DNA present in the time which it is

known to take; and probably more than this in the Hela cells. It is still a matter of speculation as to whether each of these sites represents a replicon in the sense that a genophore of *E. coli* does, each with its own regulator; and likewise it cannot yet be determined whether each replication site represents a single DNA molecule.

VIII. DNA REDUNDANCY IN EUKARYOTES

The technique of DNA hybridization has made it possible to estimate how much of the DNA in a nucleus is redundant (i.e. repetitive) in nature. Physical redundancy need not of course mean functional redundancy. Yasmineh and Yunis (1970) have found that in the mouse, 90% of the DNA is unique, while 10% is highly redundant, consisting of segments which code for repetitive sequences of polynucleotides. This 10% of the genome is the *satellite DNA*, which is present in all the chromosomes except for the Y, in the heterochromatic region near the centromere. It has an AT/GC base ratio of 1.9 (compared to 1.5 for the bulk of the DNA) and has been estimated to consist of about 1 million copies of one or a few related genes (Corneo *et al.*, 1968; Walker, 1968). However although the repeated DNA sequences are closely similar, they are not necessarily identical, which suggests that while they may have arisen through duplication, subsequent base changes may have occurred in them. Yasmineh and Yunis (1970) found that all the mouse satellite DNA was localized in the constitutive heterochromatin (Chapter 2). Part of the satellite DNA probably represents the nucleolar organizer. This has been estimated to comprise 0.1-3% of the genome of various organisms, and to consist of repeating genes coding for ribosomal RNA (Wallace and Birnstiel, 1966). The function of the remainder of the redundant DNA is not clear, but it is not hard to see reasons why certain cell proteins might need to be manufactured in amounts which would demand many identical DNA segments to produce sufficient mRNA for rapid synthesis.

The amount of satellite DNA varies considerably between different mammals. In the calf it forms as much as 40% of the genome. In man on the other hand, it only constitutes 2.5% (as already mentioned). In man, as in mouse, the satellite DNA is located in the constitutive heterochromatin, and appears to be mainly in the centromeric regions of the chromosomes (Arrighi and Hsu, 1971). The long arm of the Y chromosome also seems to be mainly composed of redundant DNA.

§ 6 §

The Fine Structure of the Nucleus and the Chromosomes

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I. INTRODUCTION

Any modern hypothesis concerning nuclear and chromosomal structure must start with the premise that the eukaryote nucleus contains nucleoprotein (which in man represents 1-2 m of Watson-Crick molecule) which is at all times arranged in an orderly fashion, whether or not this can be seen. Any model or plan of what this arrangement may be must be capable of explaining any visible structure within the nucleus, in any cell of the species under consideration, at any stage of development or activity. It must also explain any visible structure seen in metaphase chromosomes, when the nuclear membrane has disappeared, and the nucleus is not present as such; and finally it must explain any function which these structures, and other components of the nucleus which may not be visible, are believed to perform. Therefore it should be possible to explain the appearance of the interphase nucleus, of chromosomes at

any stage of mitosis or meiosis, the chromatin of the sperm head, and so on, in terms of modifications of one basic arrangement of the nucleoprotein.

Since investigations on a very wide range of organisms indicate that the basic structure of DNA, and the way in which it produces its genetic effects, are both, broadly speaking, common to all living matter, it is also desirable that any model of nucleoprotein arrangement should be capable of explaining specialized types of structures found in plants and in animals other than mammals. Two types of specialized chromosomes are of particular interest in this respect, because their large size has shed light on a number of structural problems. These are the lampbrush chromosomes of amphibian oocytes, and the polytene chromosomes of dipteran insects.

II. LAMPBRUSH AND POLYTENE CHROMOSOMES

A. Lampbrush Chromosomes

The nuclei of amphibian oocytes are exceptionally large, and the chromosomes within them are correspondingly large. In newts and salamanders the chromosomes of first meiotic prophase may be up to 900 μ in length—almost a millimetre—and are the longest chromosomes which are known. They have a characteristic appearance which has caused them to be given the name of lampbrush chromosomes. The appearance is shown diagrammatically in Fig. 33. Under the light microscope each half of a meiotic bivalent appears as a row of chromomeres, each about 1 μ in diameter, and with a pair of loops projecting from it on either side. The chromomeres can be shown to be connected by a continuous thread, like beads on a string. The places where the two halves of the bivalent cross are thought to be the sites of chiasmata.

The whole chromosome is considered to be a continuous structure, as indicated by the diagram of a single loop on the right of Fig. 33. Experiments with enzymes indicate that the central axis of the whole structure is made of DNA (which may be continuous from end to end) and that in the loops this is coated with RNA and protein. The loops appear, in fact, to be sites of RNA synthesis, and since one end of a loop is more heavily coated with RNA than the other, it has been concluded that synthesis starts at one end of the loop and moves along to the other (Callan, 1963).

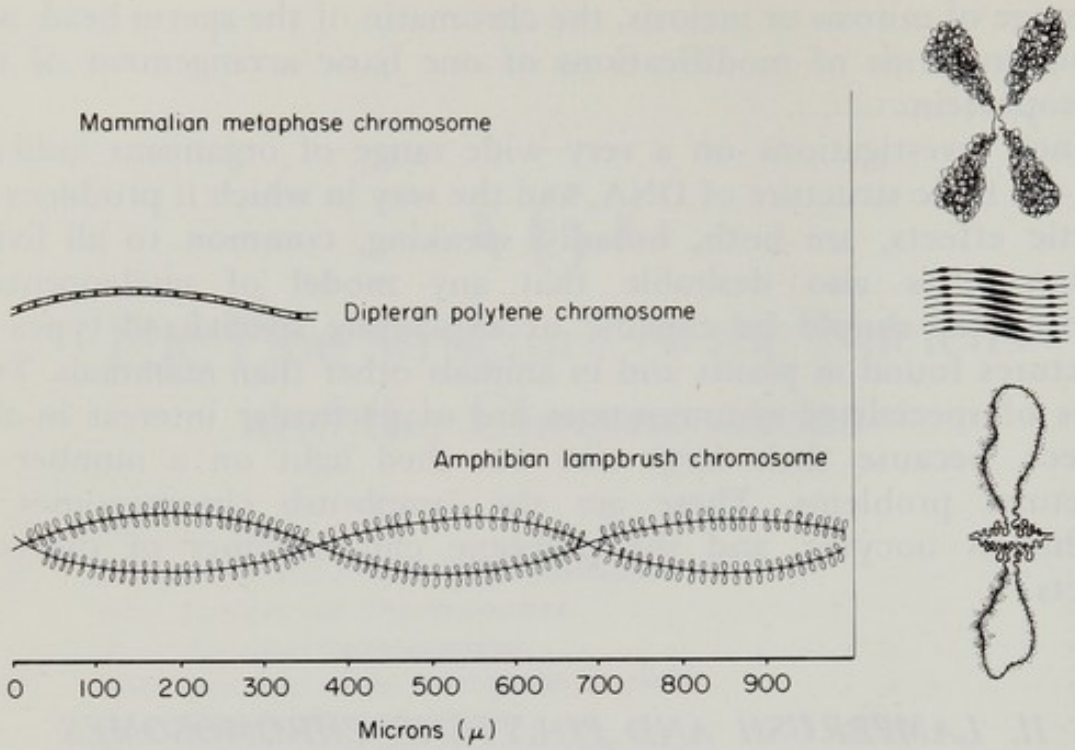

Fig. 33. *Left.* Diagrams of a mammalian metaphase chromosome (top), a dipteran polytene chromosome (centre) and an amphibian lampbrush chromosome (bottom), drawn to the same scale. *Right.* Diagrammatic enlargements of these chromosomes to show putative structure. *Top.* Mammalian metaphase chromosome according to the folded-fibre hypothesis. *Centre.* Part of polytene chromosome showing many-stranded structure; parallel thickenings (or coilings) give rise to the banded appearance. *Bottom.* A single pair of loops from a lampbrush chromosome, to show how loops may be composed of a single strand of DNA; the dots represent RNA synthesized; the coiling at the base of a loop gives the appearance of a chromomere.

Since the lampbrush chromosomes are only meiotic prophase chromosomes of exceptional size, it is tempting to hypothesize that all meiotic prophase chromosomes have a comparable structure; and as will be seen later, electron microscopic studies tend to support this view.

B. Polytene Chromosomes

The so-called polytene chromosomes are also very large chromosomes which are found only in specialized cells of the salivary glands and certain other sites in some dipteran insects, notably the fruit-fly *Drosophila,* in which most of the work on these chromosomes has been done. They are giant chromosomes, which may be over 100 μ long, and are usually joined together in a special arrangement by their ends. They are many times wider than ordinary metaphase chromosomes, and show a characteristic banding pattern

along their length, as described in Chapter 1, and shown diagrammatically in Fig. 33. The bands can be shown to correspond in order and position to genetic loci in the chromosomes, and clearly represent constant structural features.

Because of the enormous size of these chromosomes, which gives them a DNA content which may be of the order of a thousand or more times greater than in a normal metaphase chromosome of the same insect, it is thought that these chromosomes are made up by the repeated duplication of a single, very extended and threadlike chromosome, to give as many as 2048 parallel and identical threads lying alongside each other. From this comes their name (polytene means manythreaded). The bands presumably represent places where the threads are thicker, and where many threads, being all thicker at the same point, produce a visible structure. The thickening may be due to some degree of coiling of the thread at these sites rather than to a simple increase in diameter.

The polytene chromosomes have been greatly and successfully exploited for genetic studies and chromosome mapping in *Drosophila.* However there do not appear to be any comparable duplications of chromosomes which occur in vertebrates (certainly in visible form) although polyploidy does appear to be a mechanism of differentiation in some animal cells (such as those of the liver). The cells which contain polytene chromosomes are, of course, highly differentiated, and incapable of further division.

III. FINE STRUCTURE OF THE INTERPHASE NUCLEUS OF MAMMALS

A. General Considerations

The only structures visible to the light microscope in the interphase nucleus with any constancy are chromatin and the nucleolus (Chapter 2). Chromatin usually appears as a coarse network, denser in some regions of the nucleus than in others, and with a high nucleic acid content (as gauged by Feulgen staining). Its appearance in sectioned material is very much an artifact when the sections are prepared by the conventional methods of light microscopy, since the fixatives normally used cause both clumping and shrinkage of the material. Material which has been fixed, embedded and sectioned as for electron microscopy, and then examined under the light microscope, gives a more realistic picture of what is present *in vivo,* but little can be seen of the chromatin when this is done.

Indeed, if the resolution of the light microscope is compared to the probable order of size of any DNA-histone molecular complex it is at once obvious that the light microscope will not be capable of shedding light on such macromolecules unless they are present in massive parallel arrays (as in polytene chromosomes). The size relationships are as follows:

Resolution of the light microscope: 0.2-0.3 μ (depending on the wavelength of the light used; UV light will give about twice the resolution of ordinary light). (1 μ is 1/1000 mm).

Diameter of the Watson-Crick double helix: about 30 Angstrom units (Å). (1 Å is 1/10,000 μ.)

Thus even if a Watson-Crick molecule has its diameter increased a few fold by the addition of protein to its surface, it is at least 1-2 orders of size (10-100 times) below the resolution of the light microscope. However the electron microscope, with a practical resolution of 5 Å or less, should be capable of making it visible.

It can be said straight away that electron microscopic studies both of chromosomes and of the interphase nucleus have proved a disappointment; at least, they have failed to give the quick answers to problems of nuclear structure which many workers at first expected. This is not least due to technical difficulties, which have led to the dilemma that different techniques of preparation lead to different appearances, which at first sight appear to be mutually inconsistent.

Although electron microscopy of living material has been achieved, the technical difficulty is so great that nuclear structure cannot yet be usefully studied in this way; the difficulty resides in the problem of obtaining extremely thin preparations of living matter, protected from vacuum drying by thin layers of some material which allows the passage of the electron beam. In practice therefore, all biological material has to be completely dehydrated before electron microscopy is possible. Two principal methods are available; examination of material which has been fixed, embedded and sectioned extremely thinly (the thinness of the sections can be gauged from the fact that a typical sized nucleus would afford about 100 average-sized sections); or examination of unsectioned material from cells, which has been made thin by causing it to spread on a flat surface, usually during the drying process.

B. Structure of Thin-sectioned Nuclei

The only consistent structures seen in a thin sectioned nucleus are a more or less evenly distributed array of dots or short lengths of

fibre, approximately 100 Å or slightly less in diameter, which represent aggregations of the electron stain or fixative which have been bound or deposited in a linear array (Wolfe, 1969); and the nucleolus, which appears as a collection of granules in a dense mass of rather variable outline.

It is now generally considered that these fibres mostly represent the basic DNA-histone complex, or at any rate the interaction of some part of this with the heavy metals used in either fixation or staining, which form the actual electron-opaque shadow which is visualized. In some areas of the nucleus the fibres appear more densely clumped. It has been shown that these areas incorporate tritiated thymide during interphase (Hay and Revel, 1963) and that they correspond to areas in adjacent thick sections, made for light microscopy, which stain by the Feulgen technique. It can be inferred therefore that they may be equated with the interphase chromosomes. However it must be remembered that there is a good deal of protein in the nucleus which is not conjugated with nucleic acid; this may account for some of the interphase nucleus "fibres". There is unfortunately no specific electron microscopic stain for DNA, though one or two stains, such as the uranyl ion, and indium trichloride, may be considered selective for it. These enhance the staining of the thin fibres, and support their identification as DNA-containing structures; but it is still impossible to say to what extent these fibres are artifactual, and to what extent they represent structure which is present *in vivo*.

C. Structure of Isolated Fibres from Interphase Nuclei

Gall (1963, 1966) devised a technique for isolating fibrils from interphase nuclei (which has since been extensively used on metaphase cells as well). Unfixed cells are burst and their contents dispersed by placing them on the surface of distilled water, or a hypotonic solution. The combined effects of hypotonicity and the spreading forces at the air-water interface spread the cell components widely on the surface of the water, and they are then picked up on electron-microscopic grids.

The second component of Gall's technique consists of a special method of drying, the so-called "critical point drying" method (Anderson, 1951). If the grids are simply allowed to dry in air, the passage of the meniscus over the surface of the grid, with the consequent force of surface tension, results in clumping of cell constituents with loss of their individual identity. The critical point drying method obviates this by immersing the wet grid in liquid

carbon dioxide under pressure; this is then passed through the critical temperature for the gas, which causes almost instant change from the liquid to the gaseous state, without the passage of a meniscus, and thus avoids the clumping artifacts due to surface tension. As well as interphase "chromosomes", the metaphase chromosomes and some cell organelles such as microtubules, ribosomes and cell and nuclear membranes can be studied by this technique.

Gall found, by using this method, that the interphase nucleus contains fibrils of considerable length. Initially he described these as being 400-600 Å in diameter, with a 150 Å core. Other workers (reviewed in Wolfe, 1969; DuPraw, 1968) have considerably extended these observations, and it is now generally agreed that the typical interphase fibre which can be isolated from mammalian cells is 230-250 Å in diameter. The original greater diameter described by Gall was due to thickening of the fibres produced by contamination from the electron beam. No evidence can be seen of any internal structure within the fibre, although if it is assumed that the fibre contains double-stranded DNA, the latter must be highly coiled within it.

IV. THE STRUCTURE OF THIN-SECTIONED CHROMOSOMES

A. Mitotic Chromosomes

In thin section produced by the usual methods (such as fixation with osmium tetroxide or aldehydes, and staining with uranyl or lead ions) a chromosome appears rather as one might imagine a section through a piece of cotton wool (Fig. 34). A dense mass of fibrils can be seen, varying in diameter from the 20-30 Å expected for DNA to as much as 500 Å, but usually of about 100 Å or slightly less. The fibrils can, as a rule, only be followed for short distances, and often appear to be branched. Apart from this amorphous mass of fibrils, no organized structure can be seen which is intermediate in size between them and the outline of the chromosomes themselves; at the edge of the chromosomes the fibrillar network ends sharply, with no visible boundary (except that in metaphase chromosomes of some species double-layered lengths of nuclear membrane can sometimes be seen along the edges of the chromosomes, particularly at their ends).

It might be thought that it would be possible to follow fibrils through serial sections, and so determine their configuration. However the technical difficulty of obtaining many serial sections for electron microscopy is considerable (though not insurmountable)

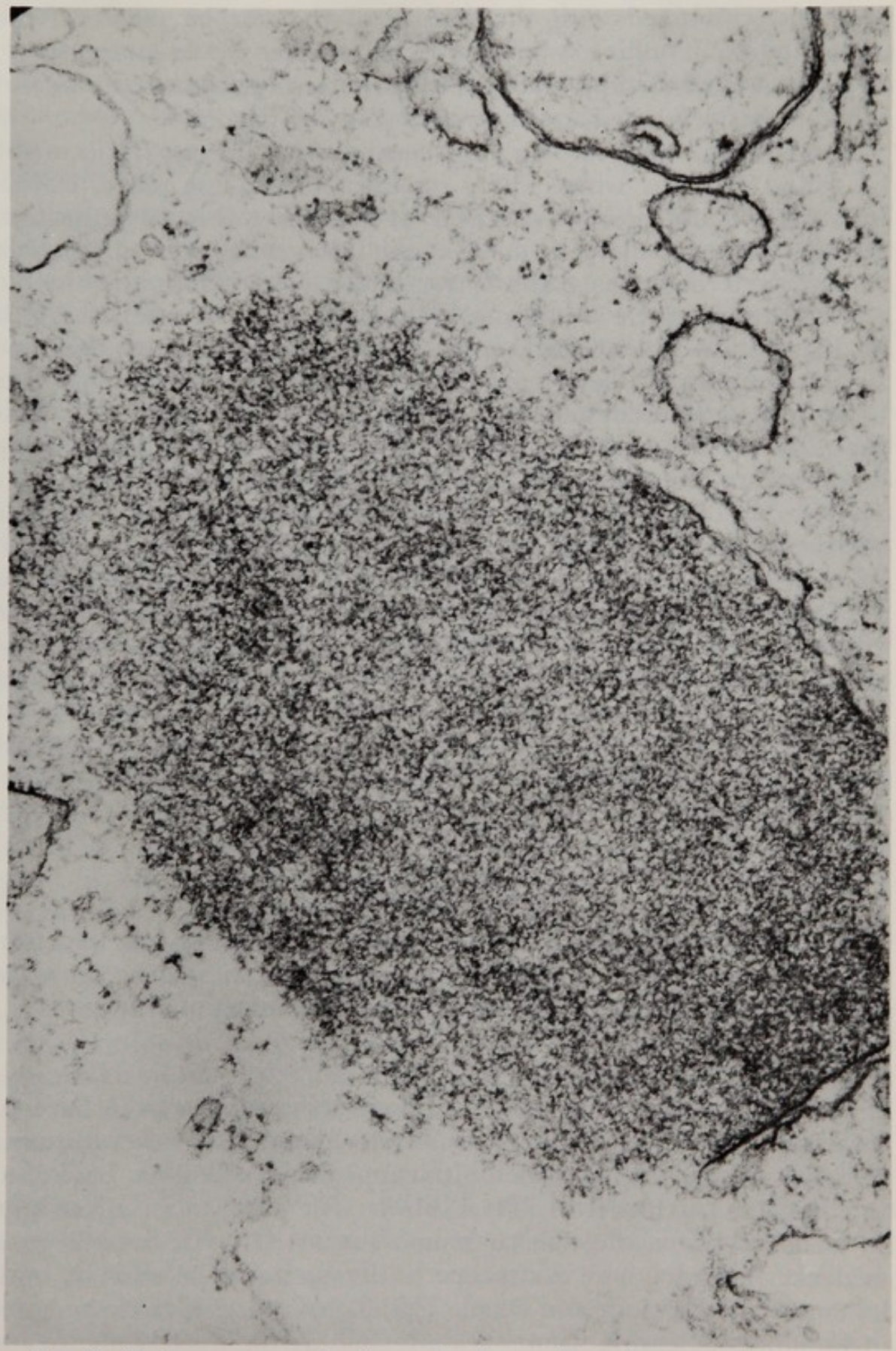

Fig. 34. Electron micrograph of a sectioned metaphase chromosome. Relics of the nuclear membrane can be seen to the right. x60,000.

and this, combined with the density of the fibrillar network, the impossibility of telling one fibril from another by its morphology, and possible distortion of the fibrillar pattern at the cut surface of sections, has precluded any successful study of this kind.

It might be expected on *a priori* grounds that thinner fibrils might be found in areas where DNA replication is taking place. Unfortunately it is not possible precisely to determine sites of replication in the nucleus at electron microscopic level, since autoradiographic resolution is inadequate for this purpose in electron microscopic work. However there is evidence in the protozoan *Euplotes,* in which distinct bands of replication pass along the macronucleus, that fine fibrils, considerably less than 100 Å in diameter, can be found in these regions (Phegan and Moses, 1967; Ringertz *et al.*, 1967). This supports the idea that the 100 Å fibres represent the DNA-histone complex, and that this dissociates or unwinds before replication.

The only region of the metaphase chromosome where a distinctive structure can be seen in sections is the centromere (kinetochore). This appears as a dense core, 200-300 Å in diameter and up to 0.1-0.2 μ in length, surrounded by a less dense zone 200-600 Å wide (Brinkley and Stubblefield, 1966). The dense core appears to consist of a pair of axial fibrils 50-80 Å in diameter, which may be coiled together. The less dense zone consists of microfibrils which loop out at right angles to the dense core, giving an appearance somewhat reminiscent of lampbrush chromosomes, or of the synaptonemal complex (*q.v.*).

B. Meiotic Chromosomes

Meiotic chromosomes are of particular interest to electron microscopists because, unlike mitotic chromosomes, they show (at first meiotic prophase) a number of visible organizational features.

1. Leptotene

At the leptotene stage the chromosomes can be seen to have an axial core. This consists of a densely staining linear structure some 200-400 Å in diameter, which often appears to be double. Lateral to this lies the usual mass of 100 Å fibrils. The axial core is often seen to be attached to the nuclear membrane at its ends; there is good evidence that each core is attached to the nuclear membrane by both its ends, and Comings and Okada (1970) have shown that the cores of homologous chromosomes are attached to the nuclear membrane close to each other.

2. Zygotene

At zygotene the cores approach each other and start to form a remarkable structure, the synaptonemal complex. When this process is complete, the nucleus has reached the pachytene stage.

3. Pachytene

The synaptonemal complex (it was called the synapt*i*nemal complex until recently, but Moses (1969) considers the new name to be etymologically better) was first described by Moses (1956) and has been found to be an almost universal feature of the primary spermatocytes of higher organisms. It consists of two lateral elements, which resemble the cores of the leptotene chromosomes in size and appearance, separated by a space of low electron density which varies in width with the species, but averages 800-1000 Å in diameter. In the centre of this space a central element can usually be seen; this is more delicate in structure than the side elements, can sometimes be seen to consist of two or four fibrils, and is usually 250-300 Å in diameter. Slender filaments are often seen running between it and the side elements. The side elements themselves vary in width from 200-600 Å, and often consist of a wider and denser lateral element, which may itself be double (in a plane at right angles to that in which the lateral and central elements lie) and a fine medial element. The synaptonemal complex is comparatively flat, rather like a ribbon; it is only 350-400 Å in thickness, and can often be seen to twist through 180° on one or several occasions (Fig. 35). There is good evidence that each complex (representing a bivalent pair of homologous chromosomes) is attached to the nuclear membrane at each of its ends.

There are considerable differences in the detailed structure of the complex in different organisms, particularly with regard to its size and to the complexity of the central element, but the basic configuration shows remarkable constancy throughout the plant and animal kingdoms. In some invertebrates there are found even more elaborate structures, polycomplexes, which resemble an array of synaptonemal complexes laid out in parallel.

It was originally thought that the central part of the complex represents the actual site of synapsis of the two homologous chromosomes, and that the filaments crossing from the lateral to the central elements might represent synapsing loops of DNA. However enzyme digestion studies (Nebel and Coulon, 1962; Comings and Okada, 1970) show that the whole complex is largely composed of protein; the lateral elements, central element, and the filaments

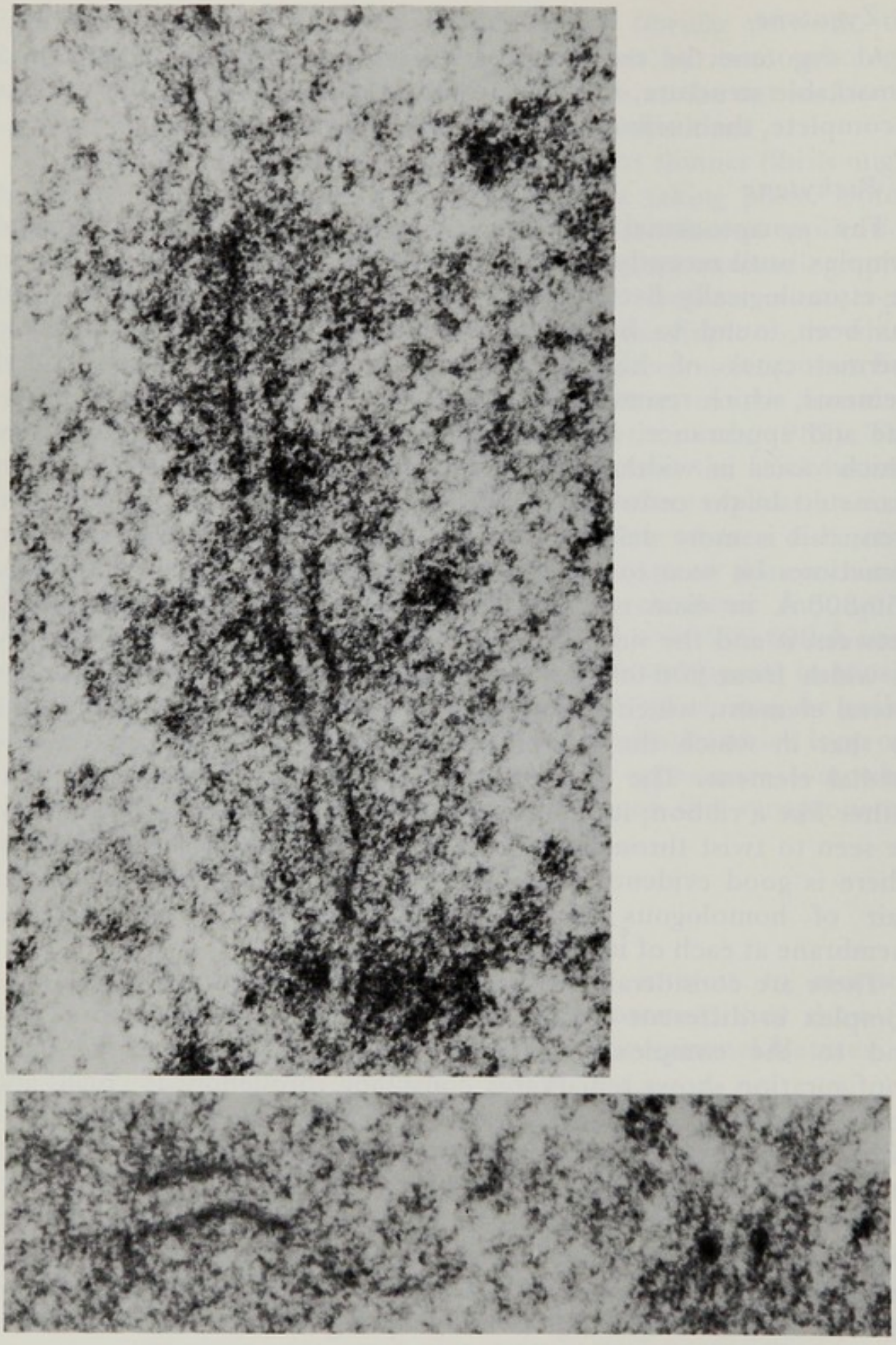

Fig. 35. Electron micrographs of part of a human primary spermatocyte, showing twisting of the complex (top) and the complex in transverse section (to right of bottom photograph). x45,000.

connecting them are all resistant to DNase but are digested by trypsin.

Lateral to the synaptonemal complex, the usual mass of 100 Å fibres can be seen; sometimes these appear to sprout out at right angles to the lateral elements of the complex in a rather regular fashion, and recent studies of the complex using a modification of the Gall technique followed by RNase digestion indicate that the fibres lateral to the complex resemble the lateral loops of lampbrush chromosomes in that they come off the chromosomes in a symmetrical fashion on either side, and form complete loops which appear to be coated with RNA (Comings and Okada, 1970; Miller and Beatty, 1969).

The fact that the complex is mainly composed of protein excludes the possibility that it represents the actual site of synapsis of the homologues, which must therefore be elsewhere (perhaps between the lateral loops). However it presumably acts as a zip-fastener mechanism which perhaps operates prior to synapsis, and facilitates it (and which cannot operate if two chromosomes are insufficiently homologous).

When the complex was first described it was assumed that chiasma formation and genetic recombination could not take place in its absence, and conversely, that the presence of a complex implied that crossing-over could occur. This argument has been of importance in considering whether or not the two sex chromosomes have a homologous segment.

It has recently been demonstrated (Menzel and Price, 1966) that a synaptonemal complex *can* exist without chiasma formation taking place. However it still seems highly unlikely that chiasma formation would occur between homologues in the absence of a complex.

4. The Fine Structure of the Sex Vesicle at Pachytene, and Its Bearing on the Problem of Partial Sex-linkage

Solari and Tres (1967) have described the ultrastructure of the human sex vesicle. It is a body about 4.5 μ by 2.5 μ, oblong in longitudinal sections and irregularly ovoid in transverse sections. It consists of a mass of chromatin fibrils which are similar in structure to those in other parts of the nucleus, but are packed differently (resembling in this the heterochromatic areas of some of the autosomes). It has no definite boundaries, the fibrils intermingling with the sparser ones surrounding the vesicle. It can be distinguished from other heterochromatic areas in the pachytene nucleus because it contains visible "cores". These cores are about 700 Å in diameter

and can be followed for considerable distances, sometimes appearing to wind around extensively.

A personal study of many human sex vesicles has shown that one of these cores forms a short loop which is attached to the nuclear membrane at both ends (Fig. 36b). This probably represents an axial element of the Y chromosome. Another core winds throughout the whole sex vesicle in a tortuous fashion (Fig. 36b) and probably represents the axial element of the X chromosome. It too is certainly attached to the nuclear membrane at one end, and probably at both, although it has never been possible to follow it through its entire length in one section.

In some animals such as the mouse (Solari, 1964), and the golden hamster (E. Ford and Woollam, 1966) it has been possible to demonstrate that the two cores come together to form a definite synaptonemal complex. This has been taken to mean that the X and Y chromosomes probably have a homologous segment in those animals.

The question as to whether there is synaptonemal complex formation between the X and Y chromosomes in man is of interest in relation to the problem of whether a homologous segment of X and Y exists, which could carry partially sex-linked genes (Chapter 3). It is still generally agreed that if a synaptonemal complex is not present, synapsis cannot occur, and the presence of homologous segments in which chiasma formation could take place is excluded. The finding, outlined above, that the complex is composed of protein, and therefore does not represent the actual site of synapsis, only weakens this argument very slightly. Solari and Tres (1967) did not find any evidence that the X and Y cores ran parallel, or that a central element or clear space was formed between them. However the author has found one or two instances, in some hundreds of sections of vesicle examined, in which two cores have run in parallel for a certain distance. There was never a clear space between them, and only in one instance (shown in Fig. 36a) was any suggestion of a central element seen.

It is not intended to suggest that these findings are evidence of the existence of homologous segments of the X and Y chromosomes in man, and in consequence, of possible partially sex-linked genes; but it would be unwise to exclude this possibility on the present evidence. The appearance of the sex vesicle changes during its period of existence, and if a synaptonemal complex was present only for a short and transitory period, it could easily be missed during ultramicroscopic examination of the sex vesicle since only small areas

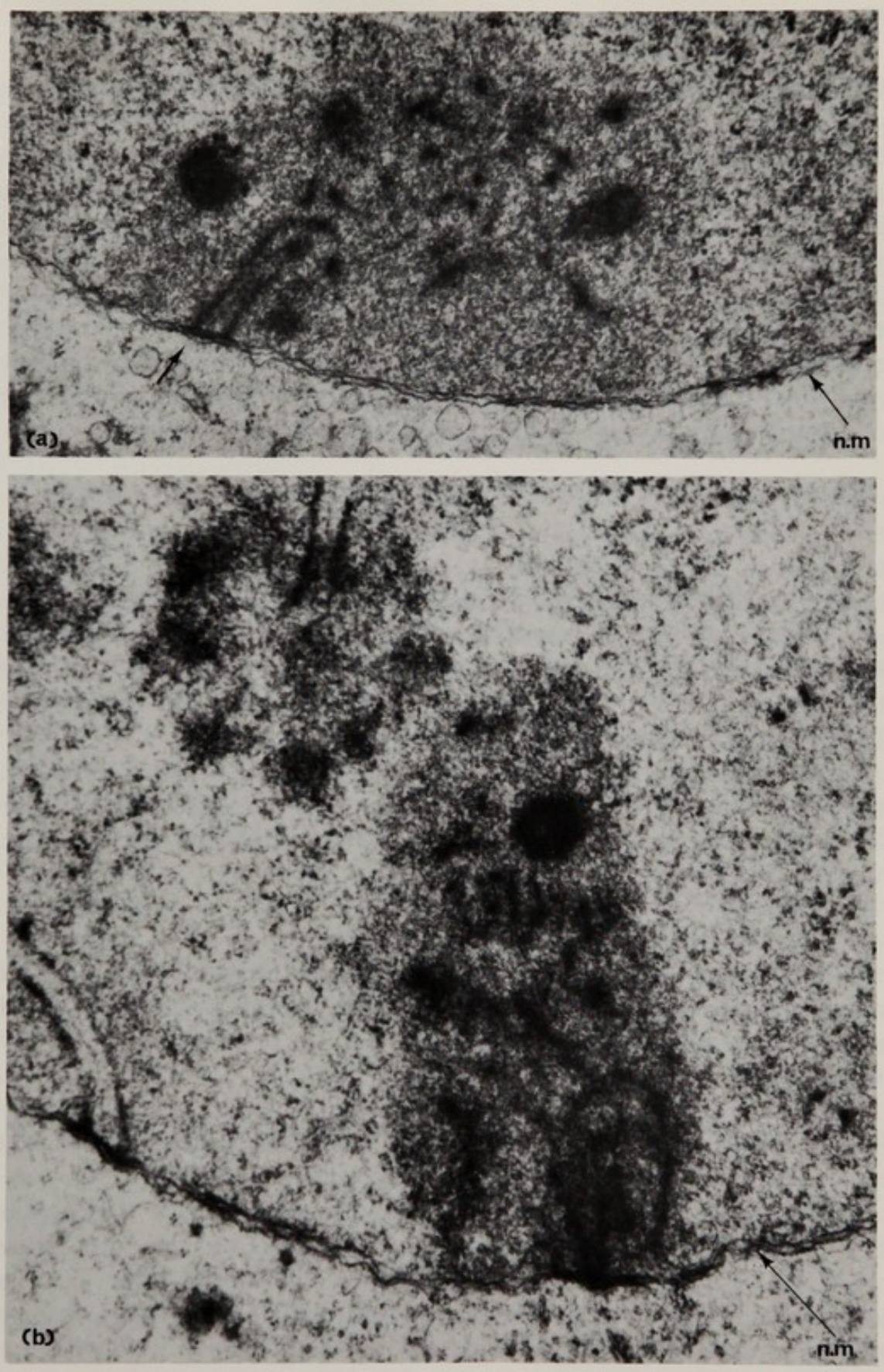

Fig. 36. Electron micrographs of the human sex vesicle. (a) Possible synaptonemal complex formation between the X and Y chromosomes, near the nuclear membrane (arrowed). (b) A complex of "cores" is visible within the vesicle. One of these makes an almost complete loop away from and back to the nuclear membrane, and may be the core of the Y chromosome. n.m. = nuclear membrane. x30,000.

of tubule (containing synchronously-progressing groups of spermatocytes) can be examined at one time. In addition to the axial filaments or cores the sex vesicle often contains a ring-shaped or dense body 0.2-0.4 μ in diameter (Solari and Tres, 1967, and Fig. 36), whose walls are formed by dense parallel fibres. The significance of this body is obscure, but the fact that it is sometimes attached to the filamentous cores suggests that it may represent a functionally differentiated part of one of them. Other structures occasionally seen at late pachytene are groups of 150 Å granules lying just inside the vesicle. These probably represent ribosomes containing RNA; RNA is found in the sex vesicle (Chapter 3).

5. Diplotene

The morphology of the sectioned chromosomes at diplotene is very similar to that at leptotene. A core is seen which consists of a double strand, each element of which is about 200 Å thick (Franchi and Mandl, 1962; Baker and Franchi, 1967) and is surrounded by symmetrical loops and columns of coiled fibres 100-200 Å in diameter, associated with clusters of large granules, and reminiscent of the "lampbrush" configuration.

In the resting, dictyate stage of female mammalian oocytes, the core is no longer visible, and only a random granulation can be seen. This perhaps represents a more diffuse lampbrush stage at which transcription can take place more actively.

6. Diakinesis; metaphase-1

The chromosomes at these stages resemble mitotic chromosomes in appearance. No organized core structure is now visible; this is also probably true of metaphase-2.

V. THE FINE STRUCTURE OF ISOLATED CHROMOSOMES

Whole chromosomes may be prepared for electron microscopy after fixation and spreading by the methods used in light microscopy, or by such methods only slightly modified (Barnicot and Huxley, 1961). Such preparations enable the complete chromosome complement of a particular cell to be examined with the increased resolution of the electron microscope, but unfortunately the only fixatives available which will allow adequate spreading (acetic-alcohol or acetic acid) seem to mat the chromatin of the chromosomes

together in such a way that fibrillar structure can only be seen at the edges of the chromosomes, and not clearly there. After hydrolysis with HCl, which is a stage in some techniques, the chromosomes appear granular. The final appearance is of a magnified version of the chromosome as seen under the light microscope, but with very little more detail to be seen (Fig. 37). The general appearance of the chromosomes (and by analogy, that of the normal chromosomes as seen in a light microscopic preparation) must be considered as a travesty of the *in vivo* morphology. In addition to normal staining, it is possible to stain such preparations negatively, or to shadow them (E. Ford *et al.*, 1968) but these procedures do not provide much additional information.

Metaphase chromosome preparations for electron microscopy can be obtained by the Gall method (DuPraw, 1965). The snag to this method of preparation is that the chromosomes can only be obtained by simultaneous rupture of a number of cells containing metaphase chromosomes, so that the complete metaphase chromosome complement of a particular cell cannot be isolated or studied; indeed it may be difficult to identify isolated chromosomes prepared in this way. However the morphology of the constituent fibres of the chromosome is much improved by this method, and the chromosomes can be seen to consist of a mass of fibres, tangled and looping in all directions, and which resemble the fibres isolated from interphase nuclei, except that they have on average a greater diameter; this is usually more than 300 Å, and perhaps relates to the less active state of the chromosomes during metaphase. DuPraw (1970) has published photographs of chromosomes from all the Patau groups, prepared by this method; and methods have been developed for calculating the dry mass of isolated chromosomes (Bahr and Zeitler, 1965). It is claimed that this can be used for identification and karyotyping (Bahr and Golomb, 1971).

Some human chromosomes prepared by this method appear to be tightly coiled up; others are partially unravelled. Unfortunately when the external morphology of the chromosomes is well preserved, it is hard to see how the constituent fibres are arranged, while when the fibres are unravelled the morphology of the chromosome is lost. Thus the precise arrangement of the fibrils within the chromosomes remains obscure, although the general principles of their arrangement can be seen, and have enabled DuPraw to propose a "folded-fibre" model for chromosome structure. This will be discussed in detail later.

Fig. 37. Electron micrograph of two human chromosomes (a No. 1 and a G group chromosome), fixed and spread as for light microscopy, and stained with uranyl acetate and lead. ×25,000.

VI. INCONSISTENCIES IN APPARENT FINE STRUCTURE BETWEEN SECTIONED CHROMOSOMES AND ISOLATED WHOLE CHROMOSOMES

The basic inconsistency in the fine structure of chromosomes prepared by these two methods is in the size of the fibrils seen. In sections the chromosomes appear to consist of fibrils of 100 Å diameter or less, whereas isolated interphase "chromosome" fibrils have a diameter of 230-250 Å, which is increased to over 300 Å in metaphase chromosomes. Which of these appearances most closely resembles the structure which is present *in vivo*?

Most investigators believe that the larger fibre is the nearest to the *in vivo* structure; but there are differences of opinion as to how this fibre relates to the 100 Å fibre seen in sections.

A. The Nature of the 250 Å Fibril

1. Internal Structure and Packing

The fibril found in the interphase nucleus and in whole chromosome preparations will be referred to, for convenience, as the 250 Å fibril (although, as already noted, its diameter is variable under different conditions). It is possible to estimate the length of 250 Å fibril which is present in an isolated chromosome by quantitative densitometry (DuPraw and Bahr, 1969). Such studies indicate that the length of the fibril is very much less than the length of the Watson-Crick molecule of which it is largely comprised, indicating that the latter must be very tightly coiled or folded within the 250 Å fibril. The ratio of Watson-Crick molecule length to fibre length has been called the "packing ratio", and has been estimated at about 56 : 1 for isolated interphase fibres, and at 100 : 1 or more for human metaphase chromosomes (DuPraw, 1970); a figure as high as 140 : 1 has been found on occasions.

DuPraw considers that a packing ratio as high as this can only be achieved by supercoiling of the Watson-Crick molecule; that is, by the formation of a coiled coil of DNA. Unfortunately it has so far proved impossible to obtain any direct evidence about the internal structure of the 250 Å fibril, because the drying which is necessary to make specimens for electron microscopy abolishes any X-ray diffraction patterns which might have been obtained from fresh material.

2. Chemistry

The 250 Å fibre has been estimated to contain 13-16% DNA, 12-13% RNA and 68-72% protein. The amount of protein is greater than might be expected in a pure DNA-histone complex (which consists of approximately equal parts of DNA and histone) and it has been suggested that the fibres as seen may contain other protein (from the "nuclear sap") which may have become incorporated into it, and partly account for its size.

3. Enzyme Digestion Studies

These suggest, as might be expected, that the 250 Å fibre has a backbone of DNA and a coating of protein. DNase breaks the fibre; RNase has no obvious effect on it; and trypsin produces a thinning; according to DuPraw (1968) it can produce a "beads-on-a-string" effect, the beads being of the same diameter as the untreated fibre, while the portions of fibre which join them may be only 30-40 Å in diameter. Wolfe (1969), however, considers that this appearance can arise by chance in untreated fibres.

4. Effects of Various Physico-chemical Treatments on Diameter

Various treatments affect the diameter of the fibres seen in isolated chromosome preparations. If chromosomes are isolated by the original Gall method, using distilled water or solutions of low ionic strength, a 250 Å fibre is seen. If the ionic strength is raised to 1 M or more, which is known to dissociate most of the protein from nucleohistone, the diameter of isolated fibres is reduced to 20-30 Å (Wolfe, 1965; Ris, 1966). If on the other hand the preparation is treated with a chelating agent, a fibre of 100 Å diameter is seen. Ris and Kubai (1970) consider that chelating agents split the 250 Å fibre into two 100 Å fibres; after treatment with such agents the fibres at first appear double; after prolonged treatment, 100 Å fibres are seen. This would explain the fact that in electron microscope sections the fibres are 100 Å in diameter, since the buffers used for the fixatives used for electron microscopic preparations are nearly all chelating agents.

However, Wolfe (1969) considers that the evidence for the dual nature of the 250 Å fibre is unconvincing, and considers that the larger fibre is produced by expansion of the smaller one when it is treated with solutions of low ionic strength. It is not yet possible to decide between these two hypotheses, since they rest on the interpretation of electron micrographs, and of what is artifact in them. The interpretations of Wolfe and of Ris are diametrically opposed.

To summarize what has been said about the structure of the 250 Å fibre; it seems clear that the basic structure of both the visible metaphase chromosome and the interphase "chromosome" is a fibre in which the DNA-histone complex is highly coiled. The fibre can have a different diameter under different conditions. It could contain two subunits, but all that is known of the behaviour and the replication of chromosomes suggests that these subunits, even if present, are unlikely to be parallel and similar in structure. Indeed, even Ris is of the opinion that if two subunits exist, they are more likely to do so as a result of folding-back or branching of a single DNA-histone complex within the 250 Å fibre than as independent structures.

VII. MODELS OF CHROMOSOME STRUCTURE

If chromosomes are composed of fibrils which contain supercoiled DNA molecules, how are these fibres organized to form the structures which are seen?

The older models of chromosome structure (Huskins, 1937; Nebel, 1939; Ris, 1957; Steffensen, 1959; Kauffman *et al.*, 1960) suggested that the metaphase chromosome consists of a bundle of parallel longitudinal strands, up to 32 or 64 in number. The more recent studies of chromosomes which have just been described do not give any visual support to such a structure; nor is it easy to reconcile a multi-stranded chromosome model with what is known of chromosome breakage and repair (Chapter 8).

Recently, by contrast, a number of models have been suggested which describe the chromosome as a tandem of single strands of Watson-Crick molecule, or even as a single molecule (indeed it has even been suggested that all the DNA in a nucleus might exist in the form of a single molecule). Freese (1958) elaborated this idea by adding hypothetical weak bonds between protein joiners which connected the tandem strands of DNA (Fig. 38a); variations in bonding might account for differences in coiling. Later modifications of this model omit the non-DNA joiners (Taylor, 1966; DuPraw, 1966, 1968). The most elaborate of these later models is DuPraw's "folded-fibre" model, and this merits detailed consideration since it not only (with some modifications) fits a number of experimental findings, but also has some direct support from the visible structure of whole chromosomes as prepared by the Gall method.

The main features of the model are as follows:

(a) The chromosome unit (or rather the chromatid unit) is a single DNA-protein fibre, repeatedly folded. It is sometimes referred

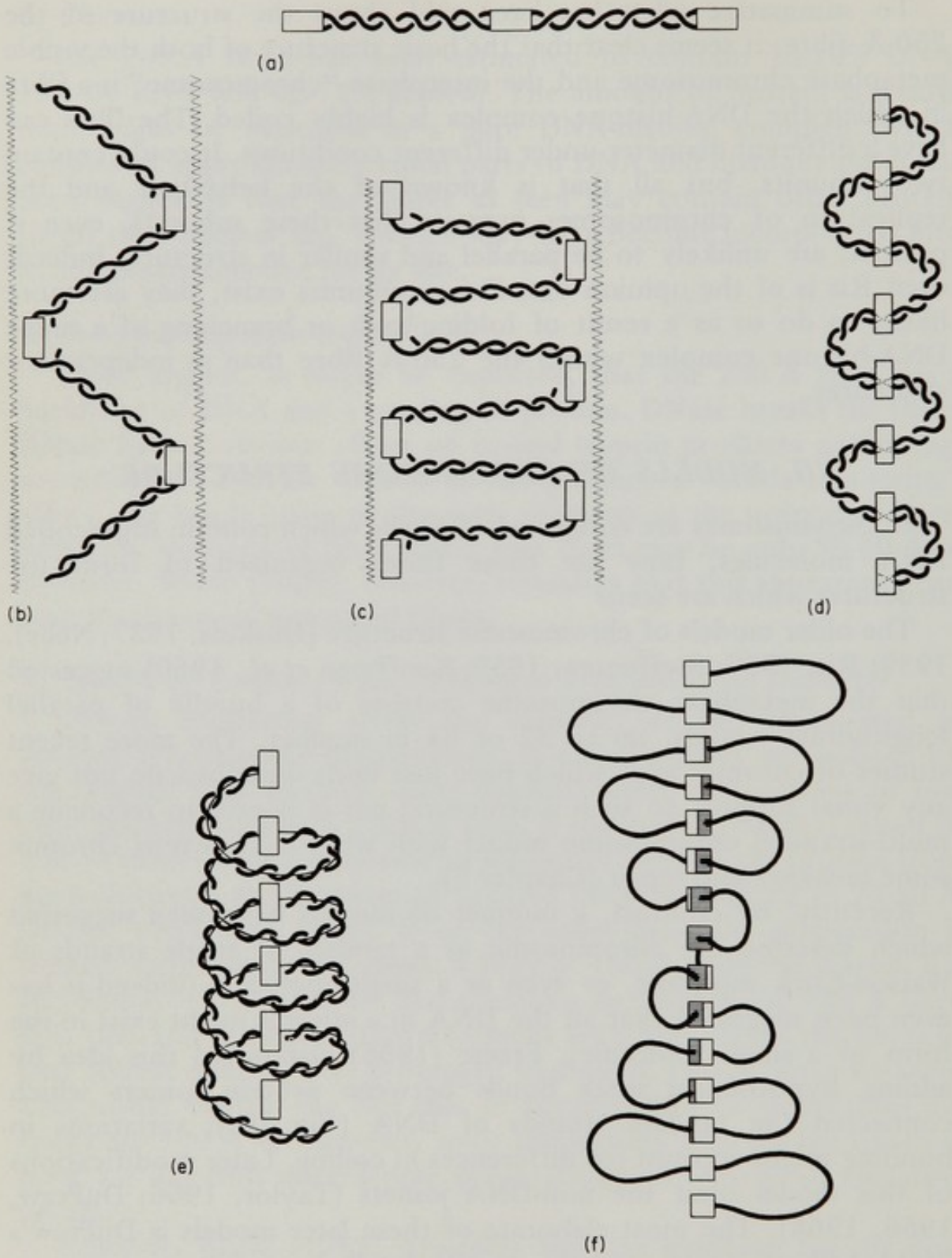

Fig. 38 (a) Chromosome model of Freese (1958). Lengths of DNA are connected by non-DNA (? protein) joiners; replication starts at these, by detachment of one strand of DNA. (b) and (c) Later modifications of the Freese model. The zigzag line represents some sort of bonding which connects the non-DNA joiners and allows for extension or contraction of the chromosome. (d) Model after J. H. Taylor (1969) in which the DNA is continuous through the joiners, which form a central axis from which spring lateral loops. (e) and (f) Elaborations of (d), to show how this model might be compatible with a "coiled-coil" (e) or a visible helical structure (f).

to as the "unit chromatid", and corresponds to one Watson-Crick molecule of DNA.

(b) This single DNA helix is wrapped in polypeptides and supercoiled to make up the 250 Å fibre.

(c) The supercoiling is induced and maintained by crosslinking of arginine-rich histones.

(d) At the S period the unit chromatid replicates at two or more replication forks.

(e) After replication the daughter fibres are held together by unreplicated portions of the original molecule, particularly at the centromere; replication here just before anaphase allows chromatid separation to take place.

(f) The fibres fold up at prophase and metaphase and in ways which are reproducible from one cell generation to the next, but not in any clearly recognizable order.

(g) As a consequence of (f) chromatids are not necessarily linear in organization; nor does the genetic telomere (end of chromosome) necessarily correspond to the cytological telomere.

The main support for the model comes, as has already been said, from the visible structure of whole chromosomes prepared by the Gall method, and particularly from such findings as that the telomere commonly appears as a series of closed loops; that terminations of fibres are seldom seen; and that a number of parallel fibres are often seen to pass through the centromere region. However it is necessary to repeat that it is difficult to see precisely how fibres are arranged in intact chromosomes prepared by the Gall technique; while in those chromosomes that are unravelled so that the fibres can clearly be seen, the external morphology leaves much to be desired.

The folded-fibre model as just described is open to certain criticisms, amongst them the following:

There is conflicting data as to whether the centromere is actually late-replicating. While replication sometimes appears to proceed from telomere to centromere, the reverse is also sometimes true. Also it can sometimes be seen that the centromere is double at metaphase, well before the chromatids separate. So the part of the model that suggests that anaphase is initiated by replication of the centromere is difficult to support.

Furthermore, the concept that the chromatid is not linear in organization may be true on a rather small scale, but it is hard to reconcile with the phenomenon of chromosome breakage and reunion with exchange of chromosome arms, which can occur

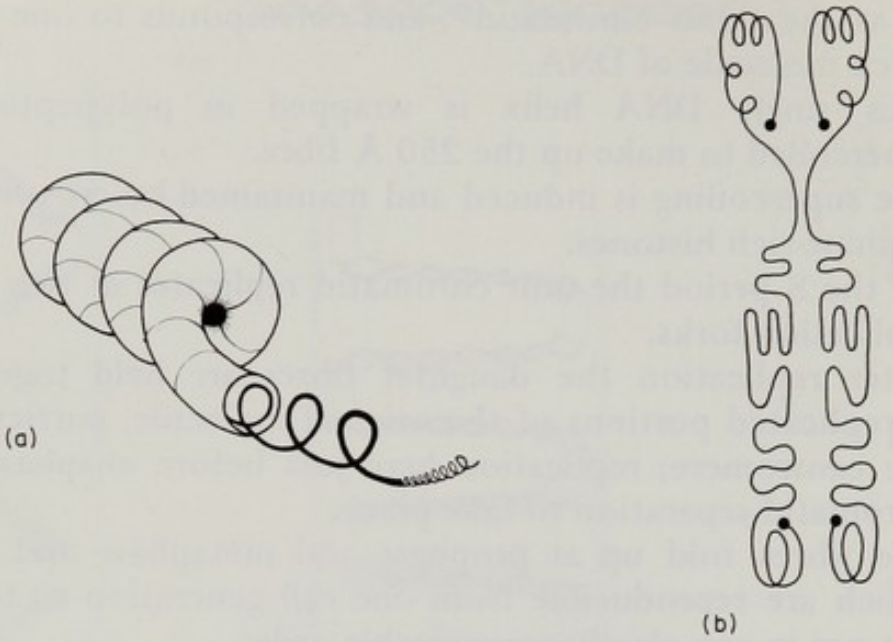

Fig. 39 (a) The visible chromosome fibre as a "coiled coil". The small coil on the right represents the DNA double helix (after E. J. DuPraw). (b) Highly simplified diagram of the "folded-fibre" model of DuPraw, showing how a single fibre (the "unit chromatid") may be folded in different ways in different regions, and how the genetic telomere (shown as a dark blob) need not coincide with the cytological telomere.

between sister chromatids during mitosis; it is also hard to reconcile this with the idea of parallel fibres back and forth through the centromere region.

However the model is *not* inconsistent (as some have suggested) with the concept of multiple replication sites in chromosomes. A single DNA molecule could easily be broken at a number of sites, and replication at these sites initiated by "primers" (Taylor, 1966). The model also fits well with radiobiological phenomena (giving a good explanation, for example, of how inversions might occur), and although it is clearly an oversimplification of the actual state of affairs it has many merits.

Taylor (1969) prefers a model in which a single DNA double helix is held into a compact form by hypothetical replication guides which are attached to, but do not necessarily interrupt, the DNA chain (Fig. 38d). This model has the advantage over the simpler folded-fibre model that it can explain the core structures seen in meiotic chromosomes between leptotene and diplotene. These certainly appear to be composed of protein at the time they take part in the formation of the synaptonemal complex at pachytene, and support the idea of some of regular protein linking structure, with loops of DNA lying lateral to it. The model also agrees with the structure of

the lampbrush chromosomes; the simple folded-fibre model is not quite adequate to explain their structure.

Taylor (1969) has in fact suggested that the lampbrush chromosome may serve as a good model for generalized chromosome structure. Since the lampbrush chromosome must be capable of subsequent modification to form a typical mitotic metaphase chromosome, this seems a reasonable suggestion; certainly any complete model for metaphase chromosome structure must also be able to explain the structure of meiotic and lampbrush chromosomes.

Recent studies on the morphology, behaviour and inheritance of the loops of lampbrush chromosomes have led Callan (1967) to suggest that the linear chromosome consists of a series of "master" loci, alternating with many "slave" copies; the slave copies give rise to the lateral loops and are corrected against the masters during the lampbrush phase, before being used for transcription. Whitehouse (1967) has further proposed that masters and slaves would have to separate before crossing-over, and then reassociate in the correct order after the completion of crossing-over. He has proposed that the slave assembly might take the form of a series of closed loops, and has proposed a "cycloid" model for this. Henderson (1971) has examined this idea critically, and has proposed models to show how such assemblies of masters and slaves might behave at meiosis.

It would be beyond the scope of this chapter further to discuss these interesting ideas about master and slave sequences; but two points may be made about them. Firstly, that a master-slave system would be of particular interest and relevance in explaining the presence and behaviour of the repetitious sequences of DNA which are now known to make up an appreciable part of the mammalian genome; and secondly that there is not as yet any firm cytological evidence to support (or to refute) these particular models.

§ 7 §

Dosage Compensation and the Lyon Hypothesis

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I. THE LYON HYPOTHESIS

The term "dosage compensation" was first used by Muller (1932) to describe the phenomenon in *Drosophila* in which both sexes appeared to show equal expression of the genes on the X chromosome, although the female had two X chromosomes and the male only one.

Owing to the late development of mammalian cytogenetics, little was known of the mechanism (or even the existence) of dosage compensation in mammals until the last decade. However the mere fact that the female mammal had two X chromosomes and the male only one implied that some mechanism for dosage compensation must exist. Hypotheses concerning the mechanism of this were formulated by several workers in the early 1960s (Stewart, 1960; Russell, 1961; Beutler *et al.*, 1962) but the clearest exposition was put forward by Dr. M. F. Lyon (1961) and her name has remained associated with the hypothesis.

The Lyon hypothesis (Lyon, 1966) is as follows:

(a) One of the two X chromosomes in the cells of normal female mammals is genetically inactivated.
(b) The inactive X chromosome can be either the maternal or the paternal one in different cells of the same animal.
(c) The inactivation occurs early in embryonic development and, once it has occurred, remains fixed throughout the further development of each cell line.

II. EVIDENCE LEADING TO FORMULATION OF THE HYPOTHESIS

The first relevant observation was the demonstration by Barr and Bertram (1949) of the existence of a sex chromatin body. They observed that a small darkly-staining chromatin body was present in the nuclei of neurones of female cats, and that this was not present in male cats. Subsequently it was shown that a comparable body could be demonstrated in the readily-available cells of the buccal mucosa in women, but not in men (Moore and Barr, 1954). This body was always attached to the nuclear membrane in these cells, unlike the position in the cat neurones, which was not related to the nuclear membrane. The sex chromatin body has since then been demonstrated in most human tissues (with the notable exception of the germ cells). Davidson and Smith (1954) showed that it takes a special form in the polymorphonuclear leucocytes, the so called "drumstick" which can be seen as an appendix to the nucleus of about 2% of these cells in women, but not in men (with the rare exception of men with a large Y chromosome; Ricci *et al.*, 1971). Apart from the buccal mucosa cells, which have been used most commonly for sex chromatin studies, the hair root is another good source of available cells, and this gives cleaner preparations than the keratinizing and often heavily infected cells of the buccal mucosa (Schmid, 1967a).

Ohno *et al.* (1959) observed that one of the two X chromosomes in the nuclei of liver cells in the female rat was dark-staining (heteropycnotic) at mitotic prophase while the single X chromosome of males was isopycnotic. This suggested that the heteropycnotic female X chromosome might be involved in formation of the sex chromatin body. At about the same time Welshons and Russell (1959) showed that mice lacking one X chromosome (39,X) are normal fertile females, implying that only one X chromosome is

necessary for normal development. It was also shown at this time by C. E. Ford *et al.* (1959) that phenotypic human females with Turner's syndrome (45,X) lacked one of the pair of X chromosomes, and also lacked a sex chromatin body.

In human embryos the sex chromatin body is first seen at the stage of the implanting blastocyst (Glenister, 1956), and according to Park (1957) it is seen earlier in the trophoblast (12 days) than in the embryo itself (16 days).

These observations suggested parts (a) and (c) of the hypothesis as outlined above. Part (b)—random inactivation of maternal or paternal X—was suggested by Lyon's observations on female mice which were heterozygous for X-linked genes determining coat colour. In such heterozygous mice these genes appeared to give rise to a "mottled" or "dappled" effect, which suggested that the pigmentation of fairly large discrete areas of skin was due to the operation of one or the other of the genes concerned, but not both (Fig. 40). A similar

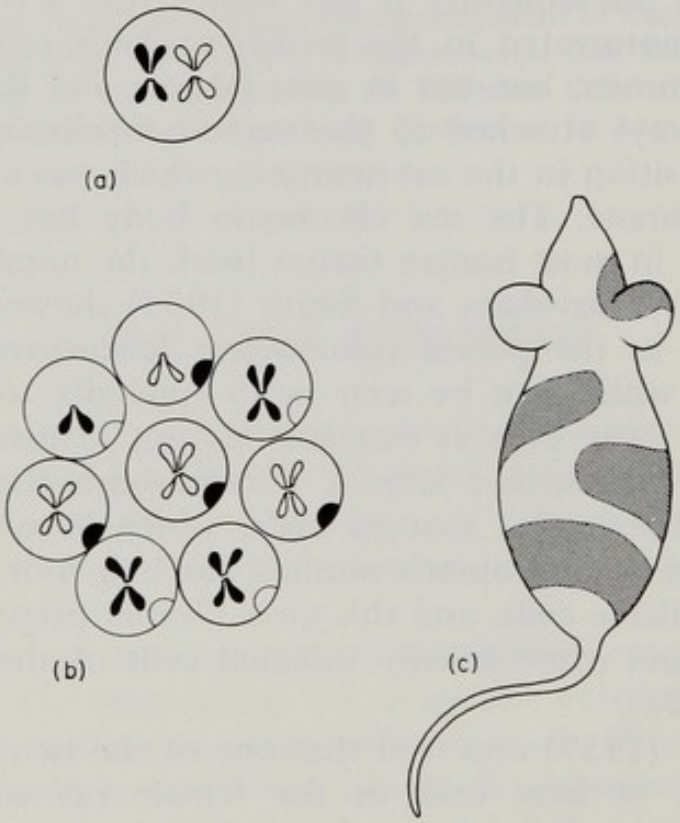

Fig. 40. The Lyon Hypothesis (diagrammatic). (a) Cell containing two X chromosomes, one bearing genes for dark coat colour (black) and the other for light coat colour (white). (b) Group of cells from a blastocyst, in each of which one or other X chromosome has been inactivated and forms a sex chromatin body. (c) Adult mouse; the dark areas consist of clones of cells descended from a single cell in which the X chromosome bearing genes for light coat colour has been inactivated; the light areas consist of clones of cells in which the other X chromosome is inactivated.

phenomenon is seen in the tortoiseshell cat. The tortoiseshell pigmentation (a mixture of black and tan patches) is only seen in female cats, and only when these cats are heterozygous for the relevant coat-colour genes.

The Lyon hypothesis was first put forward for the mouse (Lyon, 1961) but was soon extended to cover man and mammals generally (Lyon, 1962). Strong supporting evidence for X chromosome inactivation in man came from observations that individuals with an abnormal complement of X chromosomes had a corresponding alteration in the number of sex chromatin bodies present in their cells. It has already been mentioned that phenotypic females with Turner's syndrome, 45,X, lack a sex chromatin body. It was also found that phenotypic males with Klinefelter's syndrome, 47,XXY, possess a sex chromatin body (unlike normal males). Observations on individuals with large numbers of X chromosomes showed that the maximum number of sex chromatin bodies seen in their cells was always one less than the number of X chromosomes present; thus 47,XXX females had two bodies in some of their cells, 48,XXXX females had three bodies in some cells, and so on (Fig. 41). This clearly suggested that dosage compensation in such cases was effected by the inactivation of all but one of the X chromosomes present.

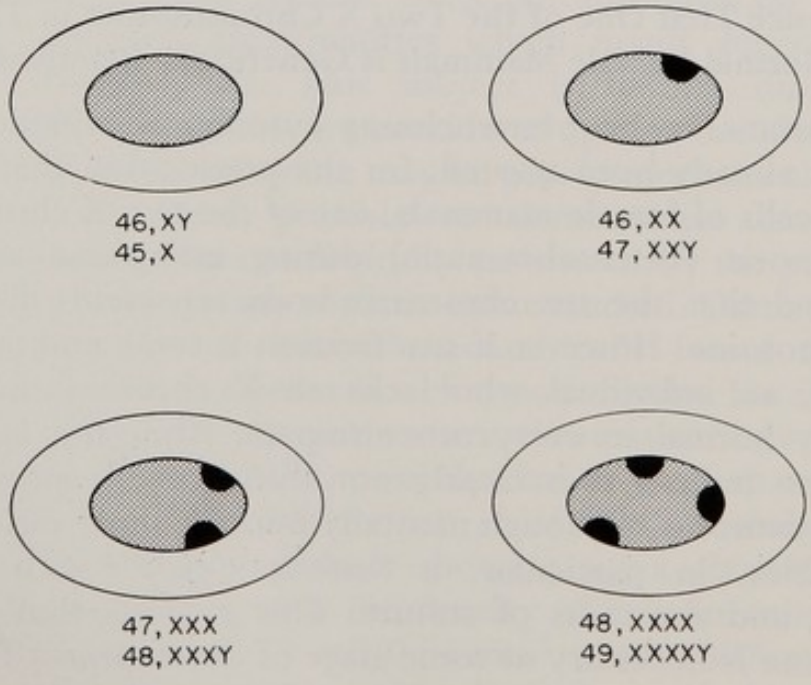

Fig. 41. Relationship between number of visible sex chromatin bodies, and sex chromosome complement. Maximum no. of sex chromatin bodies visible = Total no. of X chromosomes present −1.

Another relevant observation was made by Taylor (1960). During autoradiographic observations on the replication of chromosomes of the Chinese hamster it was noticed that one of the two X chromosomes was late-replicating in comparison with the autosomes. It was soon observed in man also that one of the chromosomes of the C-X group was late-labelling, and although this could not be identified as the X with certainty by its morphology, the presumption was very strong. It seemed likely that a late-labelling X chromosome was also the one concerned in the formation of the sex chromatin body.

III. CRITICISMS OF THE LYON HYPOTHESIS

The Lyon hypothesis has not received unanimous support; one of its strongest critics has been Grüneberg (1966). Nevertheless, inasmuch as it is possible to summarize the general feeling of the literature on the subject, there seems to be overwhelmingly more support than criticism. There are however a number of different and independent propositions contained in the hypothesis, and rather than support or condemn the hypothesis as a whole, it seems rational to consider the evidence for the various parts of it separately.

A. Evidence That One of the Two X Chromosomes in The Cells of Normal Female Mammals is Genetically Inactivated

There seems to be overwhelming cytological evidence, some of which has already been quoted, for the proposition that in many if not most cells of female mammals, one of the two X chromosomes is heteropycnotic (heterochromatic) during interphase and early in mitosis, and that the sex chromatin body represents all or most of this chromosome. However if inactivation is total, and takes place in every cell, an individual who lacks an X chromosome should be completely normal in every other respect. While this appears to be true of the mouse, it is clearly not true in man; individuals with Turner's syndrome, although mentally normal, show various physical abnormalities, in particular, a "streak ovary" with consequent infertility, and shortness of stature. This suggests that a second X chromosome is necessary at some stage of development for complete normality, at any rate in some tissues. There is in fact evidence that X chromosome inactivation does not take place in the germ cells of the ovary (Ohno *et al.*, 1962) and this might account for the

maldevelopment of the ovaries in Turner's syndrome. But the other physical abnormalities suggest that X chromosome inactivation may not be absolute in all somatic cells; minimal functional activity of a second X chromosome may be necessary at some stage of development for a normal phenotype to be achieved. However the normality of the central nervous system, which is generally the most sensitive index of slight genetic deviation, suggests that dosage compensation is complete in neurones.

On the other hand, it is also true that individuals with increased numbers of X chromosomes have a tendency to mental subnormality. While this is not always apparent in 47,XXX females, all reported men or women with more than three X chromosomes have been found to be mentally subnormal, usually severely so. Even 47,XXX females, while not frankly subnormal, may be slightly subnormal in relation to the mental capacity which have been expected from their parental background (Court Brown, 1967). It is possible however that this is not a pure genetic effect, in the sense that it is due to the direct action of additional genetic material, but that it relates to a minimal alteration in the mechanics of cell division, due to the presence of an extra chromosome. This could explain why individuals with Klinefelter's syndrome are often mentally quite normal, although they are (statistically) more commonly subnormal than persons with a normal chromosome complement. And females with an abnormally large number of X chromosomes do not show any obvious physical abnormalities which might suggest abnormalities of somatic cells. This subject is further discussed in Chapter 12, where arguments are considered against the idea that the effect of an extra X chromosome may be purely mechanical.

It has not yet been possible to demonstrate complete X chromosome inactivation cytologically (as shown by the presence of a sex chromatin body) in any somatic tissue studied. Curtis (1969) in a very careful study of buccal mucosal cells found that only $40.8 \pm 2.56\%$ of cells showed a sex chromatin body. He also found that the proportion of cells with a sex chromatin body increased during the first six days of life, possibly due to local changes in the buccal epithelium itself. Klinger *et al.* (1968) found that the proportion of cultured fibroblasts showing a sex chromatin body increased in linear fashion with the density of cells in culture (from 35-60% at cell densities of <1 cell/0.01 mm^2 to 90-100% at densities of 20-125/0.01 mm^2) and that this finding was independent of mitotic rate or rate of increase of density. This very significant finding certainly suggests that X chromosome inactivation is not

necessarily absolute or permanent in any particular cell line, but may relate to the metabolic activity of the cell.

Furthermore Klinger *et al.* (1967), working on the incidence of sex chromatin bodies during cell replication, found that the bodies were found with the same frequency in cells at the G_1 and S phases, but with a higher frequency in G_2 cells. A significant number of cells did not contain a sex chromatin body at all. Mittwoch (1967) confirmed the higher frequency with which bodies were found in cells at the G_2 phase, although Comings (1967) found the incidence at S and G_2 to be much the same. All these findings seem to point to the fact that in cultured cells there may be a significant proportion which lack a heteropycnotic X chromosome, and that the proportion can vary with the metabolic conditions. If this is true *in vitro* it may also be true *in vivo*.

Another significant finding is that where there is a structurally abnormal X chromosome, this appears to be preferentially inactivated (Klinger *et al.*, 1965). Thus where there is an abnormally large X chromosome (such as an isochromosome for the long arm) the sex chromatin body is invariably larger than normal in all cells examined, and conversely where an abnormal X is small, all sex chromatin bodies seen are small also. This finding also implies that the sex chromatin body comprises all or most of the X chromosome.

Mittwoch (1964) found that the nuclei of cells showing sex chromatin bodies were on average 6% smaller than those not showing bodies. This again suggests that the presence or absence of a visible body is not (as has often been suggested) due to artifacts of preparation, such as the way in which the cell has been flattened, but is a real presence or absence [although it could be explained by *total* absence of the X chromosome concerned; loss of an X chromosome is known to occur in ageing women (Chapter 2)].

Other findings which suggest that X chromosome inactivation is not always present have been reported by Mittwoch *et al.* (1963) and by Miller and Warburton (1968). Mittwoch found that triploid cells often have the same number of sex chromatin bodies as diploid cells, and Polani (1969) noted that most individuals reported as having a triploid, 69,XXY, cell line in their bodies had been found to be chromatin negative. Miller and Warburton (1968) found that the counts of sex chromatin bodies in cells of individuals with three or more X chromosomes differed significantly from the binomial distribution which might have been expected if the probability of a X chromosome forming a sex chromatin body was constant, and suggested therefore that this probability was not constant but varied

(perhaps in relation to the stage of the cell cycle). It seems therefore that while cells which have sex chromatin bodies can be found in any tissue (other than the gonad), it is very doubtful whether all cells have them in all tissues at all times; so that whether the inactive X is inactive all the time or not, it is probably not necessarily permanently heterochromatic.

If one X chromosome in a cell *is* permanently heterochromatic, the problem arises of what happens when the chromosome replicates; does it completely despiralize, with disappearance of the sex chromatin body during replication? Zakharov and Egolina (1968) have shown that a considerable difference in time of replication can exist in heterochromatic areas, and suggest that heterochromatin may despiralize and replicate in successive parts without disappearance of the heterochromatin as a whole; this is supported by the fact that quite densely heterochromatic areas can incorporate tritiated thymidine, and are therefore presumably in process of replication; and the findings (quoted above) that the proportion of cells showing sex chromatin bodies does not drop during the S phase, support this concept. Comings (1967) has shown that the duration of replication of the inactive X chromosome is not very different from that of the autosomes (it is actually slightly shorter) but that it both starts and ends its replication later than the euchromatic chromosomes.

B. Evidence That X Chromosome Inactivation Occurs Early in Embryonic Development and That Once It Has Occurred It Remains Fixed Throughout the Development of Each Cell Line

There is substantial evidence for the early appearance of the sex chromatin body in the embryos of all mammals which have been examined (Lyon, 1966); it usually appears at about the stage of development of the blastocyst. In man, as mentioned above, sex chromatin first appears in the trophoblast at about 12 days, and in the embryo itself at about 16 days. However there is little evidence as yet as to how universal is its appearance. No systematic spatio-temporal studies of embryos to determine over what period, and how completely, the sex chromatin appears in different cell lines, seem to have yet appeared. The suggestions in the Lyon hypothesis that X chromosome inactivation is complete, takes place over a short period, and remains fixed, lack complete cytological confirmation. The lack of inactivation which is believed to be characteristic of the germ cells may well be characteristic of other

tissues also—or inactivation may be considerably delayed in some. Clarification of this point might go far to explain the anomalies of development found in Turner's syndrome.

Issa *et al.* (1969) have found evidence in the rabbit that the late-replicating X chromosome may start to late-replicate before the appearance of the sex chromatin body in the embryo, suggesting that the two phenomena are not necessarily completely interrelated. And evidence of the fixity of X chromosome inactivation once it has occurred is rather scanty. The evidence already given in the previous section concerning the variable presence of the sex chromatin body in cells under different conditions does imply that fixity of heterochromatinization is not absolute, and although it seems likely on *a priori* grounds that once a particular X chromosome is late-replicating it always remains so in that particular cell line, it must be admitted that this is not a particularly easy proposition to prove, especially *in vivo*, although evidence will be given in the next section that it may well be true of clones of cells under certain abnormal conditions.

C. Evidence That the Inactive X Chromosome Can Be Either the Maternal or the Paternal One in Different Cells of the Same Animal (and Remain the Same Thereafter in Any Given Cell Line)

It will be recalled that the original evidence for this proposition was the supposed presence of a patchwork mosaicism for coat colour in mice heterozygous for certain coat-colour genes. Further evidence must similarly rely on the demonstration of mosaicism in heterozygotes for any X-linked gene in which heterozygosity may be demonstrable. Such demonstrations are limited by the extent of knowledge of how particular genes actually exert their effects; but broadly speaking, such genes may manifest their activity in one of three ways; by gross effects on the body as a whole; by local (and possibly demonstrable) effects on individual cells; and through purely humoral activity of the individual cells concerned (activity, that is, through their secretion, which may act locally or distantly).

Grüneberg (1966) has made a notable attack on the Lyon hypothesis in man, and collected evidence about the influence of 60 genes considered to be X-linked. Of these 60, 34 showed no evidence of their activity in heterozygotes. While this might be thought in itself to be evidence unfavourable to the Lyon hypothesis, it must be borne in mind that where genetic effects are brought about in hemizygotes by a simple deficiency of some X-linked gene, a single

dose of that gene in heterozygotes may be adequate to produce apparent complete normality. Of the remaining 26 genes, 17 might be expected to produce gross effects, five cellular effects, and four humoral effects only. Of these genes, the most significant for the argument are those which produce cellular effects (since the mechanism of action of those producing gross effects is inadequately understood, and it might not be possible to distinguish between a single and a double dose of a purely humoral effect).

The five conditions where genes manifested purely cellular effects were the following; presence of haemoglobin Xg(a+); glucose-6-phosphate dehydrogenase deficiency (g-6-p.d. deficiency); microcytic hypochromic anaemia; agammaglobulinaemia; and Hurler's syndrome (gargoylism).

Haemoglobin Xg(a+) is transmitted by an X-linked dominant gene. Heterozygotes should show two populations of erythrocytes, one Hb Xg(a+) and the other Hb Xg(a−), if the Lyon hypothesis is correct. However it has been found that in fact all the erythrocytes of heterozygotes are Xg(a+). A variety of explanations have been put forward to account for this apparent anomaly, such as the concept that this gene is not inactivated because it is placed very close to the end of the X chromosome; but a recent observation of Race and Sanger (1967) suggests that the Xg blood group may be of no use as a source of evidence. An Xg(a−) mother had a pair of twins with blood group chimaerism; the female twin was Xg(a+), and had therefore got the gene from her father's X chromosome; but the male twin was also Xg(a+), suggesting that he had somehow "caught" the antigen from his sister, and that it had become attached to his erythrocytes.

G-6-p.d. is caused by a gene (or lack of a gene) which can be shown to give rise to two cell populations in heterozygotes. Beutler *et al.* (1962) and Davidson *et al.* (1965) were able to demonstrate, by cloning cells obtained from cultures of skin fibroblasts, that pure clones of cells showing either normal or near zero activity for the enzyme could be obtained. This seemed to lend good support to the Lyon hypothesis, since each clone obtained was derived from one original cell, and these original cells should have either an active X chromosome possessing the gene or an active X chromosome lacking it, and should, according to the hypothesis, pass on this specific activity to all its descendants. Furthermore, Davidson *et al.* were able to give further support to the hypothesis by growing clones from single cells of women who were heterozygous for two different electrophoretic variants of g-6-p.d. and to show that each clone gave a single distinct electrophoretic band.

Agammaglobulinaemia and Hurler's syndrome both show what Grüneberg has called the "Beutler phenomenon" (B.P.), that is, two distinct cell types in the heterozygote, which can be distinguished from each other. While this at first sight appears completely consistent with the hypothesis, Grüneberg pointed out that the situation is not as simple as it might at first appear, and that there are two features of the B.P. which are difficult to reconcile with the Lyon hypothesis. The first of these is that the individual patches in the mosaic are very small—essentially single cells. This implies that the large patches formed as a consequence of early X inactivation must become very thoroughly mixed (and there is no embryological evidence for such a mixing process); and secondly that the B.P. is not peculiar to genes on the X chromosome; Hurler's syndrome (a mucopolysaccharidosis, in which mucopolysaccharides can be demonstrated in the affected cells) exists in two forms, an X-linked form, and an autosomal recessive form, and both of these show two distinct cell populations.

However there is now further evidence to suggest that the finding on Hurler's syndrome just quoted is incorrect. Danes and Bearn (1967) compared clones from the autosomal recessive form of Hurler's syndrome with that from the X-linked form (now usually called Hunter's syndrome) and found that all the clones from cells of heterozygotes for the autosomal form contained cells with metachromatic granules (indicative of abnormal mucopolysaccharide) while the X-linked form showed two distinct populations of clones, one showing metachromatic staining and one not.

Another X-linked condition which lends similar support to the Lyon hypothesis, and which has been described since Grüneberg's paper, is the Lesch-Nyhan syndrome (hereditary hyperuricaemia) which is due to an enzyme deficiency. Migeon *et al.* (1968) have showed that clones of cultured cells from heterozygotes for this conditions show either normal enzyme activity or no activity.

Further evidence that autosomal loci do not show the B.P. in the way that X-linked loci do was provided by Davidson *et al.* (1965) who studied heterozygotes for three enzymes showing distinct electrophoretic variants, each of which is known to be controlled by an autosomal locus. In each case clones of cultured cells derived from single cells of the heterozygote were indistinguishable from the original uncloned cell population; so that there was no evidence of random autosomal inactivation.

Still more evidence from the study of tumours supposed to have arisen from single cells in heterozygotes has been reviewed by Davidson (1970) and seems to support the Lyon hypothesis.

Agammaglobulinaemia is perhaps not a good condition for testing the Lyon hypothesis, since although the classical form of the condition is undubitably X-linked, it is also clear that the structural genes for gamma globulin are autosomal; so that perhaps this is a condition where an X-linked gene is acting as an operator gene for an autosomal locus. If this is so, a dual cell population in heterozygotes need not imply random inactivation of an autosomal locus (although this would be equally difficult to disprove).

IV. CONCLUSIONS

To summarize; Grüneberg's attack on the Lyon hypothesis has not succeeded in disproving it, and itself is open to attack because it oversimplifies the issue; he implies that to disprove one part of the hypothesis is to disprove the whole; but as was pointed out at the beginning of this chapter, the hypothesis is not one, but several, each of which is susceptible to individual consideration. However Grüneberg has certainly drawn attention to the fact that certain parts of the hypothesis are less well substantiated than others, and that there is no good evidence in man as yet of any condition comparable to the mottling in mice heterozygous for coat-colour genes; or of patchwork mosaicism on any appreciable scale. However this may still be found; for example, the pair of monozygotic female twins reported by Philip *et al.* (1969), one of whom was colour blind while the other was not, might be explained as being due to the Lyon hypothesis.

It is impossible now to uphold the Lyon hypothesis *in toto*. Evidence is lacking for early X-inactivation in every embryonic cell, for the presence of a sex chromatin body in every adult cell, and for the absolute constancy of presence of a sex chromatin body once it has appeared in a cell line. Minor variations in these respects in different tissues can go far to explain facts which appear inconsistent with the hypothesis. But there seems to be overwhelming evidence that the hypothesis is largely true for most cells in most tissues, and at most times. The hypothesis has been of immeasurable value in supplying us with a theory of dosage compensation in mammals, and in stimulating experiments to test its validity under a large number of different circumstances. Lyon (1971) has recently given her latest views on the state of the hypothesis.

§ 8 §

The Origin and Transmission of Chromosomal Abnormalities

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I. INTRODUCTION

It is usual to distinguish between abnormalities due to the gain or loss of whole chromosomes, which leads to aneuploidy (that is, a chromosome number which is different from the normal haploid or diploid number) and abnormalities which are due to structural alterations of chromosomes which lead to addition to or subtraction from their normal genetic content. Aneuploidies usually arise as a consequence of errors of cell division; structural alterations arise as a consequence of chromosome breakage followed by non- or mal-union.

II. CHROMOSOMAL ANEUPLOIDY

The main causes of aneuploidy are non-disjunction, which may be meiotic or mitotic; chromosome loss due to anaphase lagging or some other cause; and, much more rarely, errors in fertilization.

A. Non-disjunction

1. Primary Non-disjunction

If an individual is found in whom some or all of the cells have more or less than the normal chromosome complement it can be inferred that the condition has arisen as a consequence of abnormal cell division either in that individual or in cells ancestral to the cells of that individual. Usually the error arises either in the subject or in the germ cells of one of the two parents, although occasionally it may be traced back to a grandparent or even further. And usually the aneuploidy arises as a consequence of non-disjunction. This term refers to the process whereby at metaphase and anaphase of a particular cell division, either meiotic or mitotic, two homologous chromatids (or at the first meiotic division, two homologous chromosomes) fail to separate (disjoin) and pass to different daughter cells, but remain together in one of the daughter cells, with the consequence that this daughter cell has an extra chromosome, while its sister is correspondingly deficient. The cell with an extra chromosome is referred to as trisomic, and the cell which lacks a chromosome as monosomic.

According to whether non-disjunction occurs at meiosis, resulting in the formation of an abnormal gamete, or in cells of an individual after fertilization has occurred, it can be classified into pre- and post-zygotic.

(a) Pre-zygotic non-disjunction. When aneuploid gametes are produced as a consequence of this, and subsequently join with normal gametes at fertilization, individuals will be produced in whom every cell has the same abnormality, either a duplication or a deficiency of the chromosome(s) concerned. Non-disjunction can occur in spermatocytes or oocytes, and at either the first or second meiotic divisions; occasional cases are on record where it must have occurred at both the first and second divisions involving a particular gamete. It is sometimes possible to say which division must have been affected; for example, duplication of the Y chromosome can only occur through non-disjunction at the second meiotic division.

(b) Post-zygotic non-disjunction. If non-disjunction occurs in one of the dividing cells of a developing individual, and if both cell lines resulting are viable, two aneuploid cell lines will be present in that individual in addition to the normal cell line which is already present (unless the error occurs at the first cleavage division). Such an individual is called a mosaic, and is composed of a patchwork of different cell lines. Mosaicism has frequently been described in man,

most commonly as a result of non-disjunction affecting one or both of the sex chromosomes, and the variety of cell lines has sometimes made it possible to deduce that more than one non-disjunctional event must have taken place in the person concerned.

2. Secondary Non-disjunction

Non-disjunction occurring in a normal haploid or diploid cell as just described is referred to as primary non-disjunction. But if meiosis occurs in a trisomic cell, a situation arises where non-disjunction cannot be avoided; half the gametes produced must have an extra chromosome, and be themselves trisomic. This situation is called secondary or inevitable non-disjunction. It follows that a totally trisomic individual might be expected to have 50% of trisomic and 50% of normal children. In practice very few trisomic individuals are capable of reproducing, but a small series has been recorded of females with Down's syndrome (trisomy for chromosome No. 21) who have had children, and approximately half of these children have been found to be trisomic. However females who are trisomic for the X chromosome do not appear to have trisomic children (certainly no instances have yet been recorded). A possible explanation for this may be that the trisomic gamete is at a disadvantage compared to a normal gamete, and is selected against. More is said on this subject in Chapters 10 and 11.

B. Chromosome Loss

It is known in plants that single chromosomes can be lost during cell division through a process called "anaphase lagging". During anaphase, one or more chromosomes fail to move to the poles of the cell as fast as the others, with the consequence that when the nuclear membrane reforms at telophase, they are excluded from the nucleus and are subsequently lost. This mechanism can clearly result in loss of chromosomes in some cells without the consequential gain of chromosomes in others which is found after non-disjunction. Anaphase lag has not been demonstrated visually in human cells, but it seems very likely that it is the mechanism whereby sex chromosomes tend to be lost from cells with advancing age (Chapter 2).

C. Triploidy

Triploidy (a chromosome complement of three times the haploid number) has often been found in spontaneously aborted embryos,

but in pure form (i.e. affecting all cells in the body) it seems to be rather lethal in mammals, and is probably incompatible with postnatal life. Nearly all triploid human individuals who have survived after birth (and the condition is very rare) have been mosaics (or chimaeras) and have had a fair proportion of normal diploid cells in their bodies. Two triploid infants have been described who survived to a fairly advanced prenatal stage (Edwards *et al.*, 1967; Bernard *et al.*, 1967). Triploidy must arise at around the time of fertilization, either through two sperm simultaneously fertilizing a single ovum, or one sperm fertilizing two ova (or one ovum and a polar body) which become conjoined. 69,XXX, 69,XXY and 69,XYY triploids have all been described, indicating that either of these mechanisms can operate. Digyny (two female contributions) seems to be commoner than diandry (two male contributions). The subject has been reviewed by Polani (1969).

III. STRUCTURAL CHROMOSOMAL ABNORMALITIES (LEADING TO GENETIC IMBALANCE)

A. Introduction; Chromosome Breakage

If chromosomes are, as they appear to be, discrete linear arrays of genetic material, then alterations in the amount or in the order of the genetic material which they contain can only take place as a consequence of breakage followed by loss of genetic material and/or the rejoining of broken ends in such a way that the linear structure of the chromosome is altered.

The consequences of chromosome breakage have been studied ever since the classical experiments of Muller (1927) indicated that chromosomal breakage induced by X-irradiation provided a convenient and easily controllable method of studying the behaviour of breaks *in vivo*. Many agents, physical, chemical or biological, can produce breaks in chromosomes (Chapter 13), but ionizing radiations are particularly suitable for experimental purposes, partly because dose can be accurately controlled and related to the results produced, and also because they are certainly the cause of some of the chromosome mutations which occur in nature.

These studies have shown that if chromosomes are broken by ionizing radiation, various consequences can follow, which partly relate to the number of sites of breakage.

B. Single Chromosome Breaks

Simple breakage (or achromatic gaps which may lead to subsequent breakage) may be seen after irradiation of cells in interphase. If irradiation takes place before the S period of the cells concerned, the breaks occur in both chromatids of a chromosome, at the same position; if it takes place after the S period, single chromatid breaks may be seen.

If cells in which breaks have been induced are allowed to survive for a period, the broken ends of the chromosomes behave as though they were "sticky". They are unstable in the broken state, and tend to adhere to any other broken chromosome ends in the neighbourhood. Clearly the most likely outcome of this is that they will rejoin the broken end from which they were severed (possibly with some minor alteration in the sequence of the purine-pyrimidine base pairs of the DNA at the site), and this must often happen. However, an abnormal union may take place. If just one break occurs involving both chromatids of a single chromosome, the broken ends of the chromatids which are lying side by side may unite, resulting in what is called *sister strand reunion.* The consequence of this is shown in Fig. 42a. When the cell next divides, the acentric fragments of the two chromatids are lost, since they cannot attach to the spindle and thus pass to the daughter cells; and the two joined chromatids are carried by their centromeres in opposite directions, with their conjoined part forming a bridge between the two daughter cells. Before cell division can be completed, this bridge must break at some point, which is not usually precisely the middle. This results in uneven distribution of chromosomal material between the two daughter cells. When chromosome replication next takes place in the daughter cells the broken chromosome gives rise to two chromatids which have sticky ends, and which unite with each other once again. The bridge formation and breakage can then repeat itself at the next cell division. This break-fusion-bridge cycle can repeat itself through several cell generations, and each time the chromosomal material is distributed less evenly between the two daughter cells (McClintock, 1942). The ultimate outcome is usually the death and elimination of the cell line; so that sister strand reunion tends to be a self-limiting process, and does not give rise to long term genetic problems.

Muller found that the broken ends of chromosomes do not unite with the unbroken ends of other chromosomes; this led him to postulate that the end of a normal chromosome has a special structure of its own, which is stable in nature, and he called this the

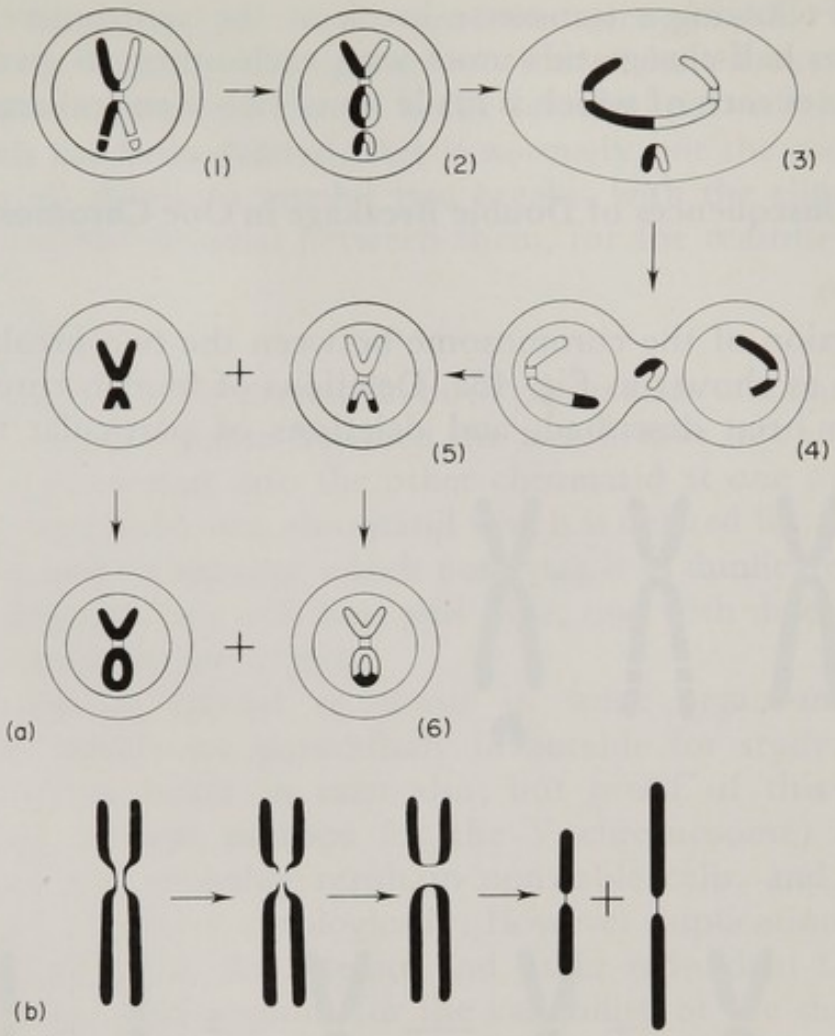


Fig. 42. (a) Sister strand reunion. Breakage of both arms of a chromosome (1) is followed by union of sister strands (2) with formation of an acentric fragment. At anaphase of the next cell division (3) a bridge is formed, which breaks during karyokinesis, so that at telophase (4) two new chromosomes are formed in the daughter cells, one duplicated and the other deficient; the acentric fragment is eliminated. The cycle repeats itself, following sister strand reunion in the daughter cells (5-6). (b) Sequence of events in isochromosome formation.

telomere. It is possible that the stability of the telomere is due to the attachment of the ends of chromosomes to the nuclear membrane.

Thus a single break in a single chromosome is unlikely to lead to serious genetic consequences, since it will either proceed to normal reunion, or it will produce an unstable cell line which will eventually be eliminated. However if more than one break occurs, either in a single chromosome or in two separate chromosomes, a variety of more serious consequences can follow.

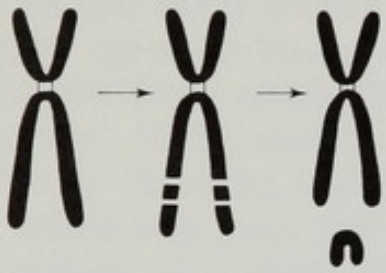
There is another possible chromosomal rearrangement which can take place following a single chromosome break, if this takes place in the region of the centromere. This is the formation of an *isochromosome*. The sequence of events postulated is shown in

Fig. 42b. Following a transverse break at the centromere, the two homologous half-chromatids unite with each other, to give two new chromosomes each of which is made up of two identical arms.

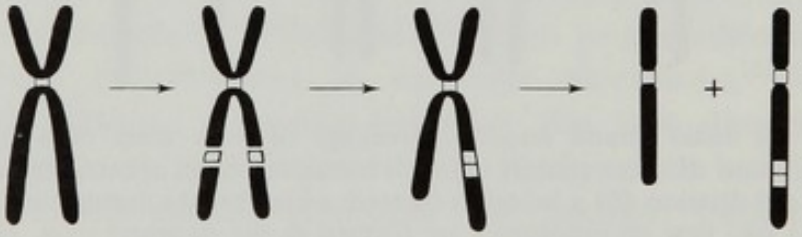
C. Consequences of Double Breakage in One Chromosome

1. Deletion

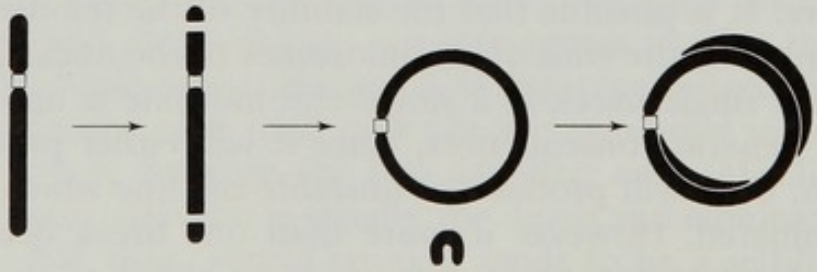
The portion of the chromosome between the two breaks may be eliminated as shown in Fig. 43a. Deletions of human chromosomes have often been described, and deletions of particular regions of



(a)



(b)



(c)

Fig. 43. (a) Chromosome deletion following a double break. (b) Chromosome duplication; following a double break in both arms the segment between the breaks (white) becomes duplicated in one arm and deficient in the other. (c) Ring chromosome formation. Following breaks at both ends of a chromosome, the two ends unite, with formation of an acentric fragment. The appearance of the ring chromosome after replication is shown on the right.

particular chromosomes may produce recognizable syndromes (Chapter 11). Although it appears at first sight on examining the chromosomes concerned that it is the terminal part of the chromosome which has been deleted, this is normally not the case, and it is likely that all deletions involve two breaks, with the elimination of the chromosomal material between them, for the reasons just given.

2. Duplication

If a double break is present in both chromatids of a chromosome, as in Fig. 43b, the region between the two breaks in one chromatid may become inserted into the other chromatid at one of the break points, giving rise to one chromatid which is deleted for the segment in question and to another which possesses it in duplicate. After the next cell division, two cell lines will arise, one with deleted and one with duplicated chromosomes.

Duplication is known to occur in some organisms (such as *Drosophila*) which are particularly favourable for study, and they almost certainly occur in man also; but proof of this is not yet forthcoming (except perhaps for the Y chromosome) since large duplications will probably result in nonviable cells, and small ones are difficult to detect cytologically. However duplication may have been responsible for the satellite and other redundant DNA in the human genome, and account for the variability of the short arms of the human acrocentric chromosomes. Where redundancy does not give rise to "genetic imbalance" in a cell, it is clearly compatible with the continued existence of that cell, at any rate up to meiosis (the effect on which will be considered later).

3. Ring Chromosome Formation

If two breaks are near the ends of a chromosome, the two broken ends may unite with each other to form a ring (Fig. 43c). Such ring chromosomes may contain nearly all the genetic material of the original chromosome, and so be compatible with the survival of the cell (particularly if it is true that the telomeric parts of chromosomes are largely heterochromatic, and relatively inert genetically). Ring chromosomes are often seen after irradiation and have frequently been described in man. They are stable at mitosis, producing a similar pair of daughter rings (unless mitotic crossing-over takes place between the two chromatids) but are unstable at meiosis (see later) if crossing-over occurs between them, and are therefore unlikely to be transmitted from parent to offspring in their original, balanced form.

4. Inversion

The part of a chromosome between two breaks may be swung round through 180° and then rejoin at its ends, with the consequence that the whole segment of the chromosome lies in inverse genetic order (Fig. 44a and b). Two sorts of inversion may be distinguished: *paracentric* (Fig. 44a) where both breaks lie on the same side of the centromere, and *pericentric* (Fig. 44b) where they lie on opposite sides of the centromere. Paracentric inversions will

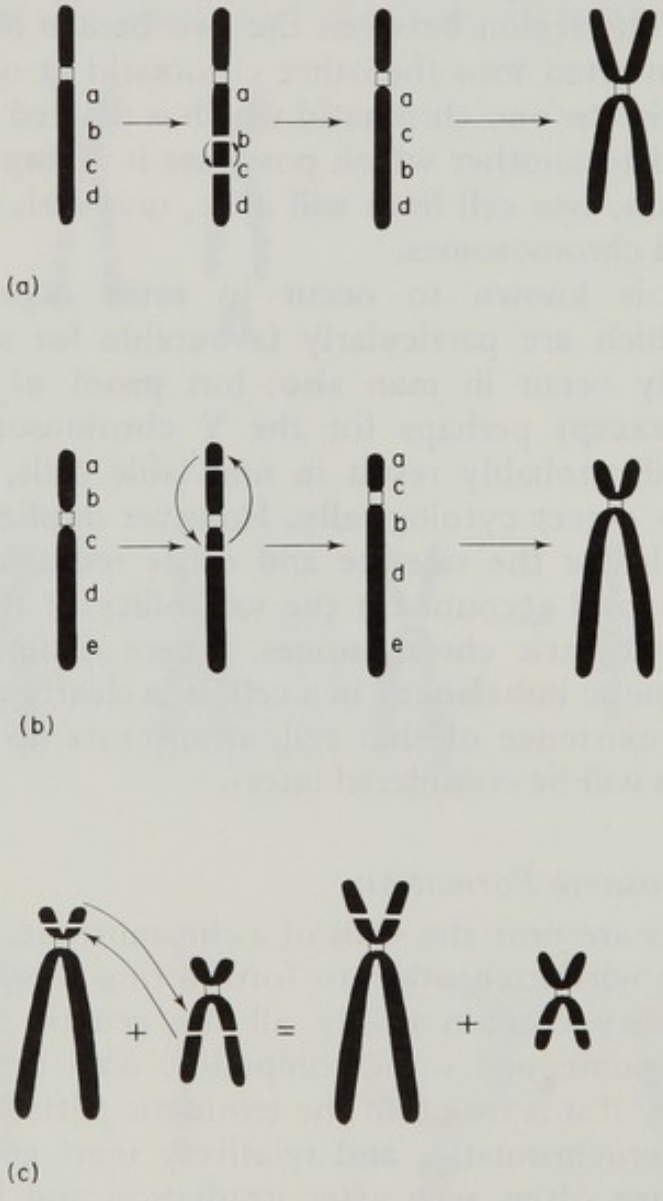


Fig. 44. (a) Paracentric inversion. Gene order is reversed in the affected segment of the chromosome, but there is no visible alteration in chromosome form or arm ratio. (b) Pericentric inversion. If the two breaks are at different distances from the centromere, inversion of the intervening segment through 180° will lead to a visible change in chromosomal morphology and arm ratio. (c) Reciprocal translocation. Following breaks in two separate chromosomes, there is exchange of part of the affected arms, resulting in two new chromosomes which are commonly different in form from the originals.

not be detectable by simple inspection of the mitotic chromosomes, since neither length nor arm ratio of the chromosome is affected; but pericentric inversions may be visible if the two breaks are at different distances from the centromere, and thus lead to an alteration in the arm ratio of the chromosome. Inversions will have no obvious genetic effect on the cells in which they occur, since the total amount and the nature of the contained genetic material remains unaltered; nor will they affect mitosis; but they do have a considerable effect on meiosis, and should be readily detected at the synaptic stage.

Pericentric inversions have been described in man on a number of occasions; paracentric have not, because they cannot be detected by mitotic studies using the usual staining methods (although the introduction of the fluorescent and pretreatment-Giemsa staining techniques may now enable paracentric inversions to be detected if they are reasonably large).

D. Consequences of Breakage Affecting More Than One Chromosome; Translocations

The term translocation was introduced by Bridges to describe the transfer of a chromosome segment from its normal position to another position. Three types at least can be distinguished; *shift*, which is the transfer of a chromosome segment to another position on the same chromosome; *insertion*, which is the transfer of a chromosome segment into another chromosome; and *reciprocal translocation* (interchange) which involves the exchange of segments between two chromosomes. The first two of these categories require the presence of at least three separate breaks; the last, only two. In addition it is probably necessary to distinguish a special type of reciprocal translocation, the so-called *Robertsonian translocation* where two acrocentric or telocentric chromosomes fuse to form a single metacentric chromosome. As the other name of this variety of translocation, "centric fusion", implies, it may involve a true centromeric fusion rather than a pure exchange (C. E. Ford and Clegg, 1969). Robertsonian translocations involving the acrocentric chromosomes of groups D and G are particularly important in human cytogenetics, and their incidence and inheritance have been reviewed by Hamerton (1966, 1968).

1. Reciprocal Translocations

Possible types of reciprocal translocation are shown in Figs 44c and 45a. The two chromosomes involved may either simply exchange

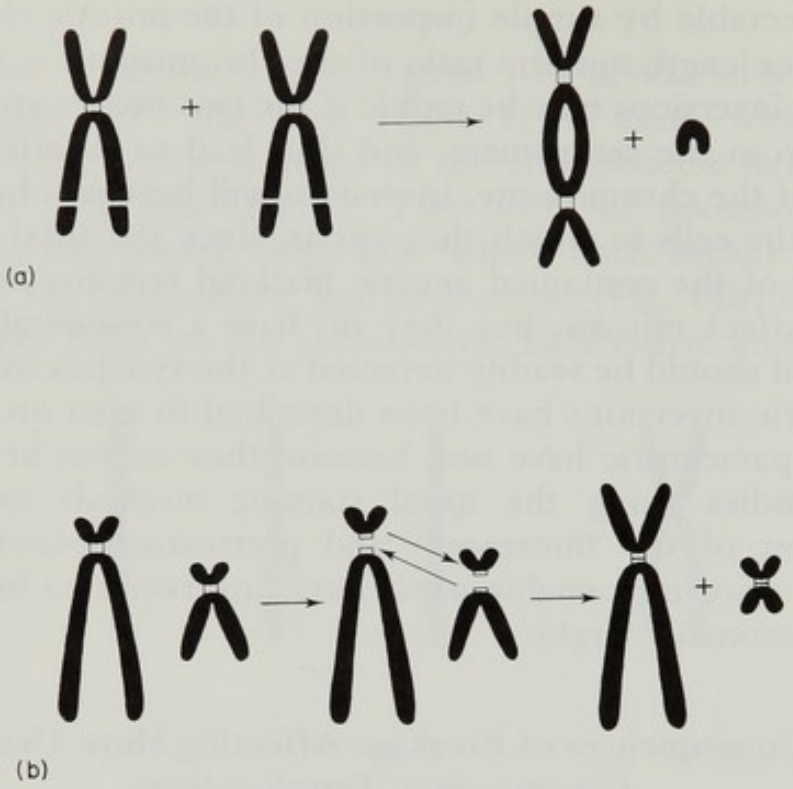


Fig. 45. (a) Dicentric formation. Two chromosomes, each with a single break, fuse to form a dicentric chromosome and an acentric fragment. (b) Centric fusion. Two acrocentric chromosomes are each broken transversely at the centromere so that short and long arms are separated. The two long arms fuse to form a new translocation chromosome, and the short arms fuse to form a minute metacentric chromosome, which is usually lost.

their acentric segments, giving rise to two new chromosomes (often with altered morphology) which contain the same total genetic material as their precursors, and will behave normally at mitosis, so that they will not affect the viability of a cell line in which they occur; or the centric parts of the two chromosomes may fuse with each other to form a dicentric chromosome while the acentric parts fuse to form an acentric chromosome. Both these latter end products are essentially unstable at cell division; the acentric chromosome is likely to be lost, while the dicentric chromosome may divide normally, but may suffer from interlocking of its two chromatids, which means either that one of them must break when the cell divides, or that non-disjunction will occur, with both chromatids passing to the same daughter cell.

Both these types of reciprocal translocation are seen after irradiation in man; the dicentric chromosomes are particularly easy to recognize, and it has been observed that the cells which contain them tend to be eliminated with the passage of time, as might be expected on theoretical grounds (Buckton *et al.*, 1962).

At meiosis reciprocal translocations give rise to complicated configurations at synapsis. These can be recognized cytologically; they may give rise to abnormal gametes of various types (see later).

2. Robertsonian Translocations (Centric Fusions, Fig. 45b)

These, as previously mentioned, represent a special type of reciprocal translocation where the breaks have occurred very close to the centromeres. It might be expected that two translocation chromosomes would be formed, one of which would contain virtually all the genetic material, while the other would consist of a centromere and two very short arms; as these short arms might largely consist of redundant DNA, possibly coding for ribosomal RNA and taking part in nucleolar organization, it might be expected that the genetic importance of these small centromeric chromosomes would be minimal, and that they might be completely lost without a significant change in phenotype.

Centric fusions of D-D, D-G and G-G types have all been described in man; indeed the first of these (D-D translocations) appears to be one of the commonest forms of autosomal heterozygosity which is found in the population at large (Court Brown, 1967). In most cases where such translocation chromosomes have been found, there is no sign of a small, largely centric chromosome in association with the translocation chromosome, so there is presumably a strong tendency for these centric fragments to be lost during cell division. Occasionally these small centric chromosomes (which may be satellited at both ends) are found in otherwise normal people, and may represent the consequence of centric fusion in an ancestor.

If small, centric fragments are lost, it makes the meiotic behaviour of centric fusions slightly easier to understand theoretically; but in practice the meiotic behaviour of such centric fusion chromosomes is not what might be predicted on theoretical grounds (see later).

3. Shifts

Shifts have not yet been described in man; indeed they would be difficult to recognize with certainty. The insertion of a segment of a chromosome into some different position on that chromosome would not produce any alteration in the total amount of genetic material, nor would it interfere with mitosis, so that the viability of the cell would not be impaired. If the shift occurred within one chromosome arm it would not be detectable cytologically (except possibly by very recently devised staining methods), but if it was from one arm to the other it would produce an alteration in the arm

ratio comparable to that sometimes seen in pericentric inversions. Indeed it is possible that some chromosomal alterations described as probable pericentric inversions might in fact represent shifts. However since a shift involves three breaks in a chromosome and an inversion only two, the latter is always more likely to be the cause of a change in arm ratio. Furthermore a shift is unlikely, for geometrical reasons, to be compatible with full synapsis at meiosis, so that any heritable alteration in arm ratio is unlikely to be caused by a shift.

4. Insertions

Insertions, like shifts, must be rarer than the other types of translocations which have been described. However, a small number of cases have been described in man which have been interpreted as due to insertions (Lejeune and Berger, 1965; Fredga and Hall, 1970). The meiotic behaviour of this type of translocation may be quite complicated, and can give rise to a variety of genetically-unbalanced gametes (Lejeune and Berger) as will be seen later (pp. 171-172).

IV. MEIOTIC CONSEQUENCES OF STRUCTURAL CHROMOSOME ALTERATIONS

In normal meiosis homologous chromosomes come together at zygotene, and synapsis takes place. While complete homology between chromosomes at DNA level can seldom if ever occur, there is evidence that a very considerable degree of structural homology is necessary for pairing to take place. If this homology is absent, synapsis does not occur, and the two chromosomes remain as univalents.

If on the other hand two chromosomes contain homologous segments, but these have altered their position in one of the pair, pairing can still take place if it is geometrically possible; but it will give rise to an abnormal configuration of the bivalent, which may be expected to be cytologically visible unless it is very small. This is easier to understand in relation to specific examples.

1. Deletions

If a deletion has occurred it is still possible for synapsis to occur between homologues one of which is affected, if the undeleted member of the pair forms a small loop of undeleted material. This has not yet been observed in man, since deletions are usually (although not always) incompatible with survival to reproductive age.

However there is evidence that they occur in, for example, a small number of cases where a parent who is mosaic for the "cri-du-chat" deletion of chromosome No. 4 has had a child affected by the deletion.

2. Duplication

As with deletions, so it might be expected that duplicated chromosomes could synapse at meiosis if the duplicated member of the bivalent formed a non-synapsing loop from the duplicated segment. However this has not yet been reported in man.

3. Ring Chromosomes

A ring chromosome may segregate normally at meiosis (and similarly may divide normally at mitosis) to yield the same ring; but if crossing-over takes place it will result in an alteration in morphology. What happens can be easily demonstrated by the following simple experiment (for the idea of which I am indebted to Mr J. S. S. Stewart). If a long, thin piece of paper has its ends stuck together with glue to form a ring, and is then cut down the middle, two rings of the same diameter will result (Fig. 46a). If the experiment is repeated, but the paper is twisted once through 180° before the ends are glued together and the cut is made, the cut gives a single ring which has a diameter twice that of the original ring. This corresponde to the *in vivo* situation where one cross-over has occurred in the ring; the resulting single ring will of course be dicentric (Fig. 46b).

If the strip of paper is twisted through 360° before the ends are joined and the cut is made, it gives rise to two rings of the same diameter as the original, but interlocked. This corresponds to the situation where two cross-overs have occurred (Fig. 46c).

Double-sized rings and interlocked rings have both been seen in the somatic cells of individuals with ring chromosomes (which is strong evidence that somatic crossing-over has occurred). However when division occurs in cells with either large dicentric rings or interlocked rings, the result must be breakage of the chromosome, with consequent uneven distribution of the genetic material between the daughter cells (rather as occurs in sister strand reunion), and the eventual product will be cells suffering from varying degrees of duplication or deficiency of the chromosome concerned. This explains why individuals who have developed with ring chromosomes may show a varying symptomatology ranging between that for trisomy and that for monosomy of the chromosomes concerned.

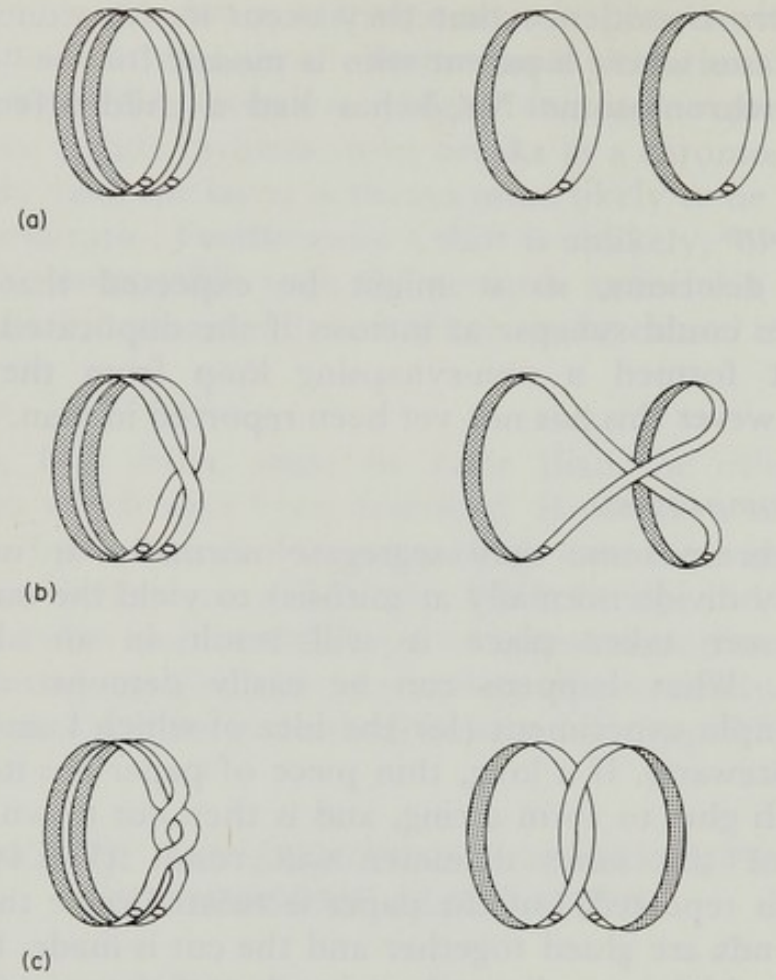


Fig. 46. Behaviour of ring chromosomes at cell division. (a) No crossing-over. Two new rings are formed. (b) One crossing-over (somatic, or after synapsis at meiosis). A single ring is formed with diameter twice the original. (c) Two crossings-over. Two interlocked rings are formed. (b) and (c) are both unstable conditions, since cell division will result in breakage of the rings, leading to uneven distribution of the genetic material between the daughter cells.

4. Inversion

Homologues, one of which contains an inversion, can pair at meiosis by the formation of an inversion loop (Fig. 47), a fact well known to experimental geneticists. The demonstration of such a loop at pachytene may be the only way in which the presence of an inversion can be demonstrated cytologically.

If crossing-over occurs inside the loop, the consequences will be different according to whether the inversion is paracentric or pericentric. Only one crossover will be considered here, although clearly more are possible if the loop is a big one.

If a single cross-over takes place in a paracentric inversion loop, the result will be that four types of gamete will be produced from each bivalent; one normal, one with the inversion, and two abnormal gametes, one of which is duplicated and dicentric, and the other

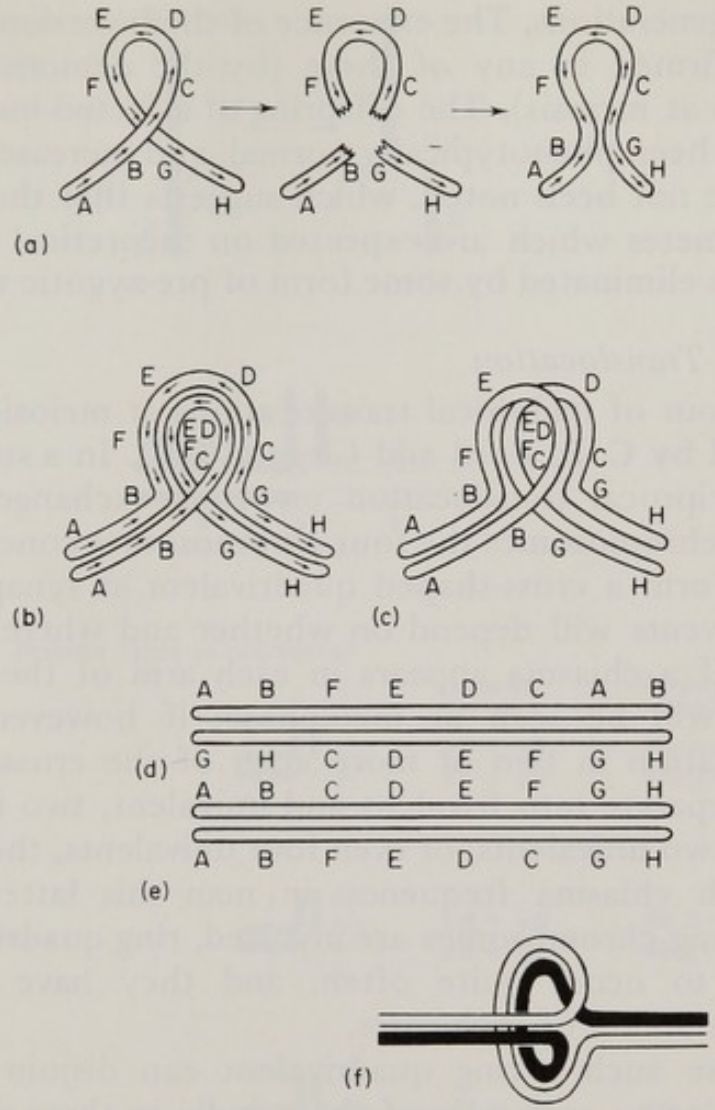


Fig. 47. Behaviour of a chromosome inversion at meiosis. (a) Possible genesis of an inversion due to a single break-causing event. (b) Formation of inversion loop at meiotic synapsis. (c) Effect of crossing-over within the inversion loop. (d) Final form of the crossover chromatids. (e) Final form of the non-crossover chromatids. (f) Common form of inversion loop actually seen. A-H, successive segments of the original chromosome.

deleted and acentric. These gametes will presumably be inviable, so the end result will be the production of gametes 50% of which are normal and 50% of which carry the original inversion.

With a pericentric inversion, on the other hand, a single cross-over in the loop will give a different product. The uncrossed chromatids will, as before, one be normal and one carry the inversion; but the crossed chromatids will both be centric, and both contain both a duplication and deletion. These could theoretically give rise to viable gametes, especially if the inversion is a small one, and these could take part in zygote formation and give rise to abnormal offspring.

A number of instances have been described in man of families in which probable pericentric inversions have been transmitted through

two or more generations. The existence of the inversion has not been formally confirmed in any of these (by the demonstration of an inversion loop at meiosis). The offspring of affected individuals have nearly always been phenotypically normal, and increased numbers of abortions have not been noted, which suggests that the duplication-deficiency gametes which are expected on theoretical grounds have probably been eliminated by some form of pre-zygotic selection.

5. Reciprocal Translocation

The behaviour of reciprocal translocations at meiosis has recently been reviewed by C. E. Ford and Clegg (1969). In a straightforward two-break reciprocal translocation involving exchange of segments between two chromosomes the four chromosomes concerned may be expected to form a cross-shaped quadrivalent at synapsis (Fig. 48). Subsequent events will depend on whether and where crossing-over takes place. If a chiasma appears in each arm of the cross, a ring quadrivalent will be seen at metaphase; if however there is no chiasma formation in two or more arms of the cross the chromosomes may separate into trivalent and univalent, two bivalents, one bivalent and two univalents, or even four univalents, though with the relatively high chiasma frequency in man this latter outcome is unlikely. If long chromosomes are involved, ring quadrivalents might be expected to occur quite often, and they have in fact been observed in a number of such cases.

At anaphase such a ring quadrivalent can disjoin so that two chromosomes go to each pole of the spindle in three different ways (Fig. 48c). These have been called alternate, adjacent-1 and adjacent-2 (McClintock, 1945). In alternate segregation the chromosomes on opposite sides of the cross proceed to the same pole; in the adjacent segregations, the two chromosomes on the same side move together. In adjacent-1, homologous centromeres separate; in adjacent-2 they move together.

These three types of segregation can give rise to six types of gamete; only the two gametes from the alternate type are balanced; one of these is normal, and the other carries the reciprocal translocation. All the gametes from the two adjacent types are unbalanced (and in the adjacent-2 variety, crossing-over between the centromere and the translocation site can give rise to four more types of unbalanced gamete). If 3 : 1 or 4 : 0 disjoining is also taken into account, the number of possible varieties of gamete is increased to 36 (C. E. Ford and Clegg, 1969).

The final result of all this is that two types of gamete which can

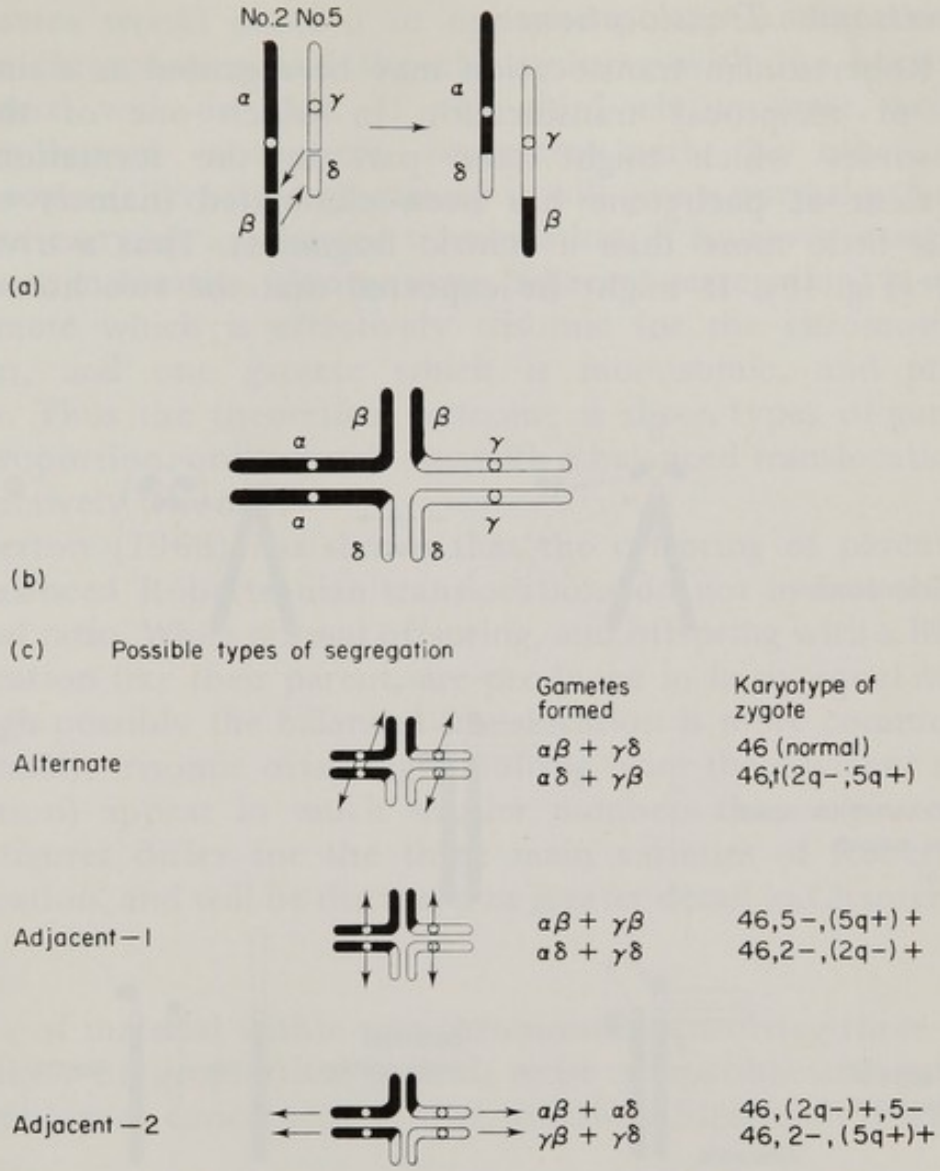


Fig. 48. Behaviour of reciprocal translocation chromosomes at meiosis (after C. E. Ford and Clegg, 1969). (a) Exchange of segments between the long arms of a No. 2 and a No. 5 chromosome. (b) Ring-of-four formation at meiosis. (c) Possible varieties of segregation, and zygotes produced thereby.

give rise to phenotypically normal individuals are produced in equal numbers; one of these is normal, and the other carries the reciprocal translocation. In addition to these, there are a large variety of other possible gametes, all of which are unbalanced and which could give rise to abnormal offspring of various types. As C. E. Ford has remarked, it is surprising how often human embryos carrying such unbalanced chromosome complements have come to term, and been born (since it might be expected that most would suffer an early death *in utero*); but this may be partly due to bias of ascertainment, due to such survivors being commonly karyotyped because of their abnormality.

6. Robertsonian Translocations

The Robertsonian translocation may be regarded as a simplified variety of reciprocal translocation in which one of the four chromosomes which might take part in the formation of a quadrivalent at pachytene has been eliminated (namely the one which is little more than a centric fragment). Thus a trivalent is formed (Fig. 49). It might be expected that the two homologous

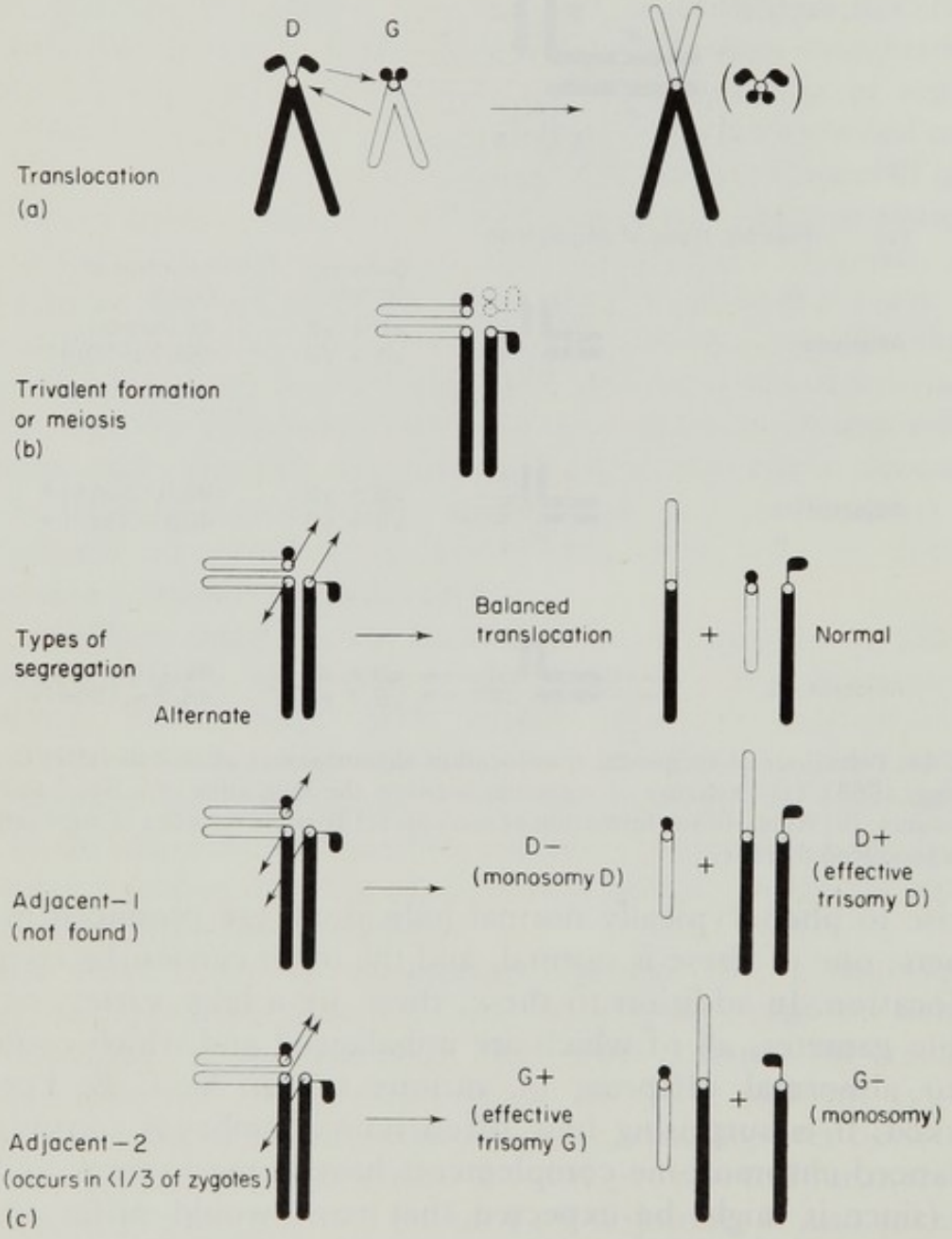


Fig. 49. Behaviour of D/G translocations (of centric fusion type) at meiosis. (a) Long arm of G chromosome translocated to position of short arm of D chromosome; small centric chromosome formed by union of D and G short arms (usually lost). (b) Trivalent formation at meiosis. (c) Possible varieties of segregation, and zygotes produced thereby.

centromeres would proceed to opposite poles, so that the translocation chromosome and the chromosome with the homologous centromere will do this. If the third chromosome, with the non-homologous centromere, segregates with the other normal chromosome (alternate segregation) it will give a normal gamete and a gamete carrying a balanced translocation; if however it segregates with the translocation chromosome (adjacent segregation) it will give one gamete which is effectively trisomic for the chromosome in question, and one gamete which is monosomic, and probably inviable. Thus the theoretical outcome is three types of gamete in equal proportion; one normal, one with a balanced translocation, and one effectively trisomic.

Hamerton (1968) has shown that the offspring of parents with such balanced Robertsonian translocations do not in fact show this expected ratio. While normal offspring, and offspring with a balanced translocation like their parent, are produced in fairly equal numbers (although possibly the balanced translocation is more common than the normal), trisomic offspring (resulting from the adjacent type of segregation) appear in much smaller numbers than expected. The actual figures differ for the three main varieties of Robertsonian translocation, and will be discussed in greater detail in Chapter 11.

7. Shifts

Shifts of material within one chromosome, involving three breaks, are unlikely on geometrical grounds to be compatible with pairing of the homologues concerned, so that univalent formation is the likely outcome.

8. Insertions

An insertion resulting from three chromosomal breaks may form a loop on synapsis, rather comparable to that which follows an inversion, except that in this instance four chromosomes are involved. Lejeune and Berger (1965) have pointed out how this may. if crossing-over occurs in the loop, give rise to unbalanced gametes with duplication-deficiencies (Fig. 50). They have also shown how it may give rise to somatic chromosomes which although they are morphologically indistinguishable from normal or translocation chromosomes may yet contain a combination of duplication and deficiency. It is a necessary prerequisite for this that the translocated material should be the same distance from the end of the chromosome in both the chromosome from which it came and in that in which it is inserted. Lejeune and Berger have called this phenomenon *"aneusomie de recombinaison"*.

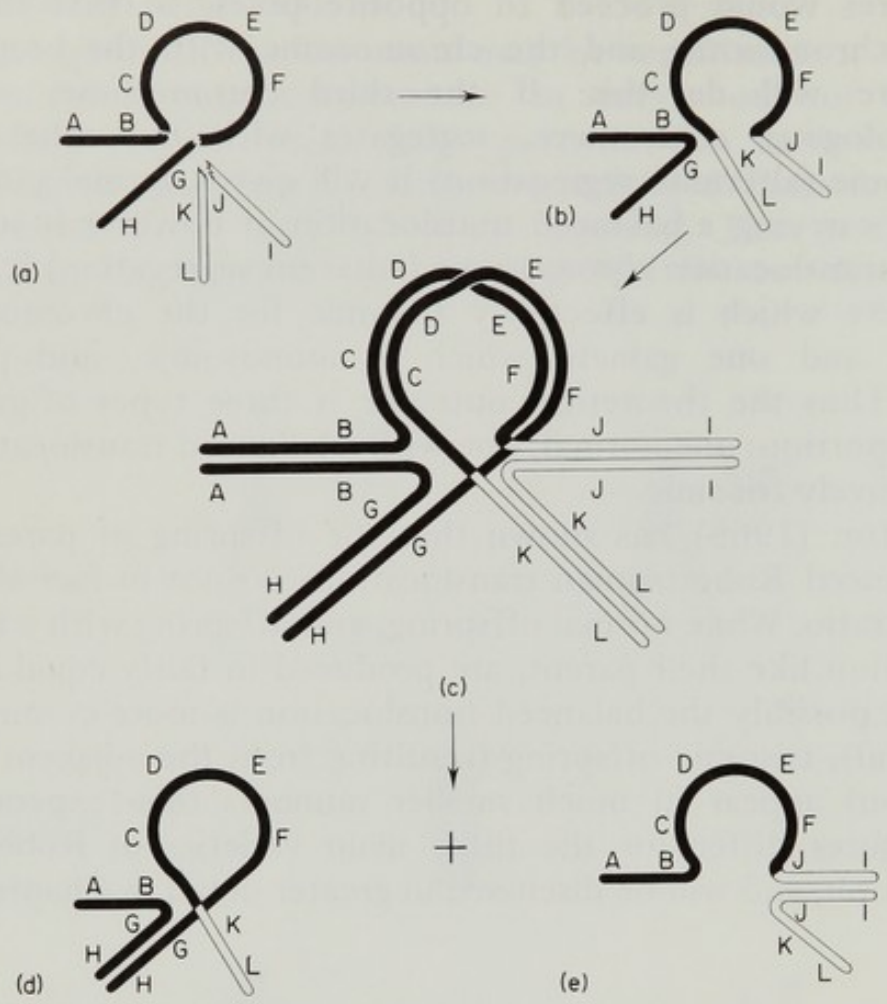


Fig. 50. *"Aneusomie de recombinaison"* (after Lejeune and Berger, 1965). (a) Breakage of a long chromosome (black) and a short chromosome (white). (b) Resulting translocation. (c) Behaviour of this translocation at meiosis. (d) Varieties of unbalanced gametes formed (in which, however, the morphology of the chromosomes may be indistinguishable from the original). A-L, segments of the two chromosomes concerned.

V. GENERAL CONSIDERATIONS GOVERNING THE ORIGIN OF STRUCTURAL REARRANGEMENTS

As a general rule, chromosome breaks are likely to occur at interphase, when the chromosomes are most extended, most physiologically active, and most vulnerable. Their geographical positions in the interphase nucleus are unknown, but may perhaps be inferred to some extent from the nature of the breakage and reunion which occurs. It is likely that broken ends of chromosomes must lie within a certain maximum distance from each other for union to take place. Various estimates have been made of this distance, ranging from 0.3 μ down to 0.1 μ (Court Brown, 1967).

Certain types of radiation, such as fast neutrons or α-particles produce a much higher density of ionization along their path than do X-rays, and it has been found that the number of two-break aberrations which they produce is proportional to dose and independent of intensity of radiation, which rather suggests that the two breaks necessary for the production of exchange aberrations are not independent, but are the result of a single hit.

It is not difficult to see how a single hit (or comparable localized disturbance) might give rise to an inversion if a chromosome is folded on itself, or even without this, if the "folded-fibre" structure of chromosomes is a reality. Nor is it difficult to see how a single hit might give rise to a reciprocal translocation, if two chromosomes are closely related topographically. Close topographical relationships almost certainly exist between the acrocentric chromosomes of the D and G groups (by virtue of their common association with the nucleolus, and their probable function in nucleolar organization) and this may explain why these chromosomes are so often involved in Robertsonian translocations. But even among the other autosomes, the frequency of involvement in translocations varies considerably, and is not simply proportional to their size. This point is elaborated in Chapter 11.

§ 9 §

The Incidence of Chromosomal Abnormalities in Embryos and Newborn Children

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I. ESTIMATING THE INCIDENCE OF RARE CONDITIONS

It is a useful statistical exercise to consider how accurately the incidence of a rare condition such as Turner's syndrome can be estimated from figures for the occurrence of the condition in population samples of various sizes. The statistical distribution which is most useful for the purpose is the Poisson distribution. This may be considered as the limiting case of a binomial distribution where the probability of an event occurring is very low.

As a practical example of the use of the distribution, let it be assumed that the *actual* incidence of Turner's syndrome is 0.4/1000 female births. If random samples of 1000 or 10,000 female births are studied, what actual numbers of individuals with Turner's syndrome are likely to be found in these samples?

Taking a sample of 10,000 births first, the *a priori* expectation is that there will be four cases of Turner's syndrome. If this number is called u, then according to the Poisson distribution the probability

that samples containing 0, 1, 2, 3, or more cases will be found is given by the series

$$1/e^u,\ u/e^u,\ u^2/2e^u,\ u^3/(2)(3)e^u,\ u^4/(2)(3)(4)e^u \ldots$$

The percentage frequency of such samples can be obtained by multiplying the probability by 100 (since probability is expressed mathematically in terms of a sample of 1). The e is of course the base of natural (Napieran) logarithms; an account of the distribution can be found in any textbook of statistics.

The percentage of samples of 10,000 in which various numbers of cases of Turner's syndrome will be found, as calculated by the Poisson distribution, are given in Table 2.

Table 2

Per cent of samples of 10,000 in which different numbers of cases of Turner's syndrome will be found if the expectation is 0.4/1000

No. of cases	Per cent of samples of 10,000
0	1.8
1	7.3
2	14.7
3	19.6
4	19.6
5	15.7
6	10.7
7	6.0
8	3.0
9	1.3
10	0.5

This table exemplifies some important points. First, if the actual incidence is 0.4/1000 births, it is nevertheless equally probable that any random sample of 10,000 will contain 3 cases as it will 4, and almost as likely that it will contain 2 or 5. So even with a sample of this magnitude it is quite easy to estimate wrongly to the extent of half or twice the true figure (more than 20% of samples will give a figure of 2 cases or less, and nearly 5% will give 8 or more). Admittedly, more than half the samples will contain between 3 and 5 cases; but a sample of 10,000 is evidently not really large enough to give an accurate estimate of the incidence of such a rare condition.

A sample of only 1000 will be manifestly inadequate for the purpose, but it is nevertheless interesting to study a similar table of probabilities for a sample of this size (Table 3).

Table 3

Per cent of samples of 1000 in which different numbers of cases of Turner's syndrome will be found if the expectation is 0.4/1000

No. of cases	Per cent of samples of 1000
0	67.0
1	26.8
2	5.4
3	0.7
4 or more	0.1

Thus the majority of samples of 1000 would be uninformative, and contain no cases at all.

For really large samples the accuracy of estimation of incidence can be tested by using the fact that the variance of the Poisson distribution is equal to the mean (the expected number per sample). Thus for a sample of 100,000 the expected number of cases of Turner's syndrome will be 40, with a standard deviation of $\sqrt{40}$. So 95% of samples may be expected to contain $40 \pm 2(\sqrt{40})$, that is, between 28 and 53 cases. Even a sample of this size might not infrequently give a best estimate of the incidence of the condition of 0.3 or 0.5/1000. But this is getting reasonably close to the true incidence.

Similar tables can be made for a condition with the frequency of occurrence of Down's or Klinefelter's syndromes; if it is assumed that the true frequency of these conditions at birth is 1.5/1000, then the percentages of samples of 10,000 which will contain various numbers of cases are given in Table 4.

In this example 95% of sample values lie between $15 \pm 2(\sqrt{15})$, that is, between 7 and 22 approximately, and 99% of sample values should lie between $15 \pm 3(\sqrt{15})$, that is between 3 and 27. Of practical significance for the cytogeneticist is the fact that more than 5% of samples will have values as low as 11 or as high as 19.

Since this is the order of size of sample which has been used to estimate the incidence of (for example) Klinefelter's syndrome, it is evident that present estimates of the incidence of this syndrome, the

Table 4

Per cent of samples of 10,000 in which different numbers of cases of Down's syndrome will be found if the expectation is 1.5/1000

No. of cases	Per cent of samples of 10,000
5	0.2
6	0.5
7	1.0
8	2.1
9	3.3
10	4.9
11	6.7
12	8.4
13	9.7
14	10.3
15	10.3
16	9.7
17	8.6
18	7.1
19	5.6
20	4.2
21	3.0
22	2.0
23	1.3
24	0.8
25	0.5
26	0.3
27	0.2

47,XXX syndrome, and particularly the 47,XYY syndrome are still tentative. The only condition due to a chromosomal abnormality in which figures for the incidence are reasonably accurate is Down's syndrome. The true incidence of the other syndromes just mentioned could be rather different from the best estimates of incidence which can be made on present data. The 47,XYY syndrome is a good example. Its incidence can only be guessed at at present. If the incidence found in the series of neonates studied by Ratcliffe *et al.* (1970), Sergovich *et al.* (1969) and Lubs and Ruddle (1970) are amalgamated (a suspect procedure since the groups concerned were ethnically different and may not have been selected in the same way), the incidence of the 47,XYY syndrome is found to be 12/6784, about 1.8/1000. Using the Poisson distribution the rough 95% confidence limits for this estimate are 5-19/6784, that is,

between 0.74 and 2.80/1000. It would be unwise, on present data, to attempt to estimate the incidence of the syndrome more accurately than this (although it must be admitted that it is surprising, if the incidence is as high as 2/1000 or more, that the condition was not more often found before 1965, in view of the large number of karyotypes which must have been made by that date; but perhaps only a minority of these were performed on phenotypically normal individuals, either newborn or adult).

Finally it is necessary to add a note of caution. The Poisson distribution is based on the assumption that the expectation that an event will occur is fixed. This is probably not so for many chromosomal abnormalities. There is evidence (Chapter 11) that some trisomic conditions may occur in epidemics, and that environmental factors may alter the expectation of their occurrence. Similarly, structural alterations of chromosomes may be induced by environmental factors, such as exposure of a pregnant woman to ionizing radiation. However until more is known about the influence of environmental factors in the causation of chromosomal disorders, the Poisson distribution offers a useful check on the reliability of estimates of incidence from samples.

II. THE INCIDENCE OF CHROMOSOMAL ABNORMALITIES IN EMBRYOS AND NEWBORN CHILDREN

A. Methodology

Many studies have been carried out on the chromosomes of aborted embryos. The method which has almost invariably been used has been the culture of the tissue of the abortus; either of the embryo itself or of its membranes. This method is not ideal, since the cultures often fail—in about 50% in most series; and it also allows for the possibility of chromosome mutation during the course of the culture. However in most cases, particularly where the embryo itself is defective or absent, there is no alternative. In occasional cases where the embryo has not been dead too long, it should be possible to obtain dividing cells direct from the haemopoietic tissue of liver and spleen, but this is seldom if ever attempted.

The results of a number of studies were collated at the Geneva conference (1966). 788 karyotypes were obtained from 1406 cultures, and 153 of these (19%) were abnormal. This figure may be compared with an incidence of only 2% of chromosomally abnormal embryos in induced abortions.

The incidence of sex (or X) chromosomal aneuploidies can be studied by examination of the sex chromatin. This has been done on some large series of consecutive births (Maclean *et al.*, 1964; Marden *et al.*, 1964) and will detect most aneuploidies involving the X chromosome. It is now possible to carry out comparable tests for aneuploidy of the Y chromosome by searching for a fluorescent Y-body in cells of the buccal mucosa or hair root, but reports on a large series are not available at the time of writing.

Other chromosomal abnormalities in neonates can only be detected at present by karyotyping a large series (this is too time consuming to have been generally adopted as a method, although computer-aided automation of karyotype analysis may eventually make it possible); or by attempting to make a complete ascertainment of cases appearing in a given population or area. Estimates made by this method are likely to be too low because not all cases may be diagnosed, except for Down's syndrome; here it is possible because of the high incidence of the syndrome, the long time it has been recognized, and the relative ease of diagnosis at birth.

B. The Incidence and Nature of Chromosomal Aberrations in Aborted Embryos

It was estimated at the Geneva conference (1966) that 15% of all pregnancies ended in spontaneous abortion. In the 50% of examined abortuses in which karyotyping was possible approximately a fifth showed chromosomal abnormalities. Since it seems unlikely that the 50% in which culture failed would have shown a lower proportion of abnormalities, it can be estimated that a minimum of 3% of all conceptions result in a chromosomally abnormal embryo which is aborted.

An analysis of the varieties of chromosomal abnormality found is given in Table 5. About 40% of chromosomally abnormal conceptuses were trisomic for an autosome; about one-third of these were for an E group chromosome (usually No. 16), one-third for a G group chromosome, one-sixth for a D group chromosome, and the remainder divided among the other groups, A, C, B and F in order of frequency. About 20% of chromosomally abnormal conceptuses were monosomic for a chromosome of the C group, and as they were usually chromatin negative it was assumed that the chromosome involved was the X. Just over 20% were triploid or (less commonly) tetraploid; about 8% were mosaics, and the remainder a miscellany of translocations, double trisomies and other abnormalities.

Table 5

Chromosome abnormalities found in spontaneously aborted embryos (Geneva conference, 1966)

Karyotype	Number found
Trisomy A1	2
A2	3
A3	2
B	2
C	4
D	9
E (mostly 16)	21
F	1
G	19
Total trisomies	63 (41%)
Monosomy (probably 45,X)	32 (21%)
Triploidy	26 (17%)
Tetraploidy	8 (5%)
Mosaicism	12 (8%)
Translocations	3 (2%)
Other abnormalities	9 (5%)
Total	153

Total cultures: 1406. Successful cultures: 788
Chromosomal abnormalities found in 153 (19%)

C. The Incidence and Nature of Chromosomal Aberration in Neonates

The incidence of some chromosomal aberrations in neonates is given in Table 6 (after Court Brown, 1967). About 1% of all newborn children may be expected to show a chromosome abnormality. Approximately half of these are structural rearrangements of autosomes (of which the commonest appears to be 46,D–,t(DqDq)+, with an incidence of about 1/800). The remainder are roughly equally divided into autosomal trisomies and sex chromosome aneuploidies. The commonest of these may be Klinefelter's syndrome, 47,XXY, with an incidence of about 2/1000, followed closely by Down's syndrome, 47,XX or XY, G+ (1.5/1000). Then come 47,XXX (1.2/1000), 45,X (Turner's syndrome, 0.4/1000) and finally the other significant autosomal trisomies, 47,XX or XY, D+ (Patau's syndrome) and 47,XX or XY, E+ (Edwards' syndrome) which together account for about 0.07/1000. Other abnormalities

Table 6

Frequency of newborn subjects with chromosomal abnormalities
(After Court Brown, 1967)

Condition	Per cent of newborn children	
Sex chromosome aneuploidy*	0.18	(Males 0.20%, 21/10,725, including 12 XXY) (Females 0.16%, 12/10,000 including 3 XO and probably 9 XXX)
Trisomy 21 (Down's syndrome)	0.15	
Trisomy D (Patau's syndrome) / Trisomy E (Edwards' syndrome)	0.07	
Autosomal rearrangements	0.50	
Total	0.90	

* Not including the 47,XYY syndrome, which would not have been ascertained by the methods used.

were too rare for their incidence to be estimated (and figures were not available for the incidence of the 47,XYY syndrome at the time these estimates were made).

Other independent estimates have been made of the incidence of Patau's and Edwards' syndromes and of the cri-du-chat syndrome, 46,Bp– (Polani, 1969). Patau's syndrome was estimated to occur in 1/7000-9000 births, Edwards' syndrome in 1/3000-7000 births, and the cri-du-chat syndrome in 1/50,000-100,000 births.

It will be realized, in the light of what was said in the first section of this chapter, that all these figures are approximations and are liable to revision when more data are available. Such revision is likely to be up rather than down, since one of the main difficulties is failure of ascertainment of rare conditions. It is also possible that environmental factors may cause certain conditions (such as some autosomal trisomies) to be commoner at some times than others.

D. Reconciliation of the Incidence of Chromosomal Aberrations in Abortuses and in Neonates; the Morbidity of Abnormal Conceptuses

If the figures given above for the incidence of chromosomal abnormalities in embryos and neonates are combined, it seems that a

minimum of 3.5-4% of conceptuses must be chromosomally abnormal (the figure may well be higher since it takes no account of failures of implantation and very early abortions which may not be detected). There is some evidence that early spontaneous abortions may have a higher rate of chromosomal abnormality than all abortions; Arakaki and Waxman (1970) found that in 127 cultures from early abortions (representing a 35.5% success rate) there were almost 50% which were chromosomally abnormal; these comprised 24 sex chromosome aberrations (including 18 45,X), 31 autosomal aberrations and eight cases of polyploidy.

Some 90% of the 3.5-4% of abnormal conceptuses are aborted, including virtually all the triploids, tetraploids, and autosomal monosomies, most autosomal trisomies (except for trisomy 21 and some trisomies 13 and 18), some mosaics and interchanges, but rather few sex chromosome aneuploidies with the exception of Turner's syndrome, 45,X. It can be calculated that for the 45,X conceptuses, 90 may be aborted for each one that is born alive, a remarkable finding which is further discussed in Chapter 10.

Probably 90% of the abnormal conceptuses will result in phenotypic abnormality (the exceptions being those structural rearrangements, balanced translocations and mosaics which are compatible with a normal phenotype).

Although the abortion of chromosomally abnormal embryos must be regarded as genetically beneficial to the human race, the amount of morbidity, both physical and psychological, amongst the mothers concerned, in a large population, must be very great. Unfortunately there is no obviously prophylactic measure available, except perhaps the avoidance of some aneuploidies due to non-disjunction by the encouragement of women to have their families while they are in the earlier part of their reproductive life.

III. CHROMOSOMAL ABNORMALITIES AS A CAUSE OF RECURRENT ABORTION

Although chromosomal abnormalities are not a common cause of recurrent abortion, they should not be ignored. Wilson (1969) studied 60 families in which there had been more abortions than normal pregnancies and found two instances of translocations (one D−,t(DqDq)+ and one 46,t(1?−; Gq+) as well as an unusually high proportion of minor autosomal variants. There will always be occasional cases of a balanced translocation which is incompatible

with a normal offspring (or sometimes, pregnancy), such as the 45,22−,t(22q22q)+ balanced translocation, which may lead to a series of abortions without a single normal pregnancy. Nevertheless it seems evident that chromosomal abnormalities are not amongst the commonest and most important causes of recurrent abortion.

§ 10 §

Abnormalities of the Sex Chromosomes

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I. INTRODUCTION: THE SEX CHROMOSOMES

A. Evolution of Sex Chromosomes and Sex Determining Mechanisms in Vertebrates

Most bisexual animals have a genetic sex-determining mechanism which is carried on their chromosomes (though in one or two invertebrates a cytoplasmic mechanism has been reported). The commonest mechanism is that one sex is homozygous and the other heterozygous for certain genetic loci. It is thought that this was initiated by the appearance of only a few genes on one of a pair of autosomes. The region of the chromosome concerned can be called the differential (or non-homologous) segment of the chromosome, while the rest of the chromosome remains similar to the homologous chromosome and is called the homologous or pairing segment.

If the differential segment is small it may not be possible to distinguish one sex chromosome from the other by either size or morphology. This is in general true of fishes, amphibia and reptiles; though even when it is so it may be possible to demonstrate a genetic difference between the two sex chromosomes. For example, it is possible in the axolotl by hormonal treatment to produce a sex-reversed female which will produce sperm, mate with another female and produce offspring. Three-quarters of these are found to be females. The best explanation of this is that maleness in the axolotl is determined by homozygosity for certain genes, which act in effect like recessive genes. The female is heterozygous for these genes, and when two females mate and produce offspring, only one quarter of these are homozygous for the male-determining genes.

Mammals and birds, both descended from the reptiles, have opposite sex-determining mechanisms. In mammals the female is the homozygous sex, possessing a pair of X chromosomes, while the male is heterozygous, having an X chromosome and a (normally) smaller Y chromosome which determines the male sex. In birds the male is homozygous, with two equal sex chromosomes, while the female has one of these (and probably a minute second sex chromosome).

By the time that there is a major size difference between the two sex chromosomes it follows that the differential segment is large and the homologous segment smaller (or even absent). A homologous segment can be demonstrated in a number of mammals, but there has been controversy as to whether it exists in some others, and especially in man, and the position is not yet resolved (see Chapter 3), although it is clear that if man does possess a homologous segment it must be very small.

B. The X and Y Chromosomes in Mammals

It is clear that if the X and Y chromosomes become very different from each other in size (and therefore presumably in genetic content) there will be genetic problems arising from the presence of a double dose of certain genes in one sex and a single dose in the other, and this will necessitate some mechanism for *dosage compensation* in order to neutralize any deleterious effects which may be thus caused. The way in which this appears to be brought about is very different for the X and Y chromosomes, although equally effective. Ohno *et al.* (1964) have observed that male-determining factors appear to be closely clustered on the Y chromosome, while female-determining characters are diffusely located on the X chromosome. This concept

(which perhaps suggests that the Y chromosome was the one upon which differential genes first appeared) has enabled the mammalian Y chromosome to develop as a chromosome containing a small cluster of genes determining maleness, and a larger remaining part which is largely genetically inert, and composed of heterochromatin. The X chromosome on the other hand, because it contains a fairly large number of structural genes, spread throughout its length, must be involved in dosage compensation early in its evolution; and the mechanism of this is the early inactivation, during embryonic development, of one of the pair of X chromosomes (discussed in Chapter 7).

Ohno *et al.* have also observed that the X chromosome seems to form the same proportion of the haploid chromosome complement (about 5%) in nearly all mammals. There are occasional exceptions; for example, the golden hamster has about twice this proportion of X-chromosome, the creeping vole three times, and the field vole four times; but these large X chromosomes probably arise as a result of translocations involving the addition of autosomal material, or through reduplication of the X chromosome itself; in the field vole it is found that in the female, all of one X chromosome and three-quarters of the other become heterochromatinized an extreme example of dosage compensation; for each diploid nucleus in either male or female must have exactly one functional X chromosome; no more, no less.

There is also some evidence that the X chromosome may carry similar genes in different mammals. For example the genes for haemophilia A and B, X-linked in man, are also X-linked in the dog and probably in the horse. And the gene for anhydrotic ectodermal dysplasia, which is X-linked in man, is also X-linked in cattle. By contrast with the X chromosome, the Y is very variable amongst different mammals, both in size and morphology and in the amount of heterochromatic material contained. This implies a certain dispensability of much of its genetic content. As with the X, so with the Y, a large chromosome suggests either a large amount of heterochromatic or redundant material, or the presence of translocated autosomal material.

It is important to remember that the genes determining sexual characters need not be and indeed are not all situated on the sex chromosomes. Autosomes may also carry such genes, and the sex chromosomes may merely carry genetic mechanisms which trigger off autosomal genes determining sexual characteristics at certain stages of development. Such autosomal genes may play a part in

cases where phenotypic males are found to be cytogenetic females, and *vice versa*.

C. The X and Y Chromosomes of Man

The morphology of the human sex chromosomes has already been described (Chapter 2). The relative genetic inactivity of the Y chromosome and of the inactive X chromosome in women is reflected in the fact that both are heterochromatic. But the heterochromatin they contain is of different sorts. The heterochromatin of the inactive X is facultative; it appears (in most cells) early in embryonic life, although not in the germ cell line, it does not stain so intensely with fluorochromes (Chapter 2), and it presumably exists as a mechanism for dosage compensation. The heterochromatic part of the Y chromosome on the other hand, which forms much of the long arm, is constitutive heterochromatin; it appears probably in the latter part of embryonic life, is present in the germ cell line and in the sperm head, and it shows intense fluorescence with fluorochromes.

1. The X Chromosome

The X chromosome is known on genetic grounds to bear a large number of genes, some dominant, some recessive and some intermediate. These X-linked genes will be considered later in relation to chromosome mapping (Chapter 14). However one X-linked gene is worthy of special mention at this stage, because it can shed light on the genesis of many aberrations involving the X chromosome. This is the gene for a variant of haemoglobin, haemoglobin Xg. Because it is a dominant gene, and thus can be detected in a heterozygote, and because it is very common, occurring with a frequency of about 66%, it can in many cases be used to show from which parent a supernumerary X chromosome is derived.

In normal women the genes on one of the two X chromosomes are inactivated in somatic tissues, according to the Lyon hypothesis. However if dosage compensation were complete in woman, it would be expected that the 45,X phenotype would be indistinguishable from the normal. In the mouse the XO phenotype is like this, and the XO mouse is both phenotypically indistinguishable from the XX mouse, and fertile. But the 45,X woman is both infertile and phenotypically different from the normal woman, so that dosage compensation is clearly incomplete, at any rate in some tissues.

2. The Y Chromosome

If the Y chromosome were completely inert genetically, it would be expected that 45,X individuals would be phenotypic males, and that 47,XYY individuals would be indistinguishable from normal males. Since neither of these suppositions is correct it is clear that the Y chromosome contains some active genes. These genes determine maleness—in particular the formation of a functional testis in which spermatozoa are produced. Although phenotypic males have been described with a 46,XX karyotype, and with apparently normal male genitalia, no such male has yet been described in whom normal spermatogenesis occurs.

Apart from sex-determining genes, no other genes have been located with certainty on the Y chromosome. There is reasonable evidence that a gene determining the development of a hairy pinna of the ear may be carried on the Y chromosome; the trait is common in some parts of India, and is transmitted to all the sons and none of the daughters of men who have it (it is always difficult to be absolutely certain that such a condition is not sex-limited rather than sex-linked), which is a prerequisite for any Y-linked gene. No other unambiguous instance of a possible Y-linked gene has yet been described (though there have been a number of conditions put forward as possible candidates, none of which have withstood rigorous examination). Indeed it is likely, from what has already been said about the Y chromosome, that Y-linked genes would not be expected to have any marked genetic effects.

II. THE SYNDROMES OF SEX CHROMOSOME ANEUPLOIDY

The main syndromes will be considered in approximate order of frequency in the neonatal population (and hence in relation to their clinical frequency and importance).

A. The 47,XXY Syndrome

This syndrome is commonly called Klinefelter's syndrome. Klinefelter *et al.* (1942) first described a syndrome "characterized by gynecomastia, aspermatogenesis without A-Leydigism, and increased excretion of follicle-stimulating hormone", to quote the title of the relevant paper. It was shown in 1956 (Bradbury *et al.*, 1956; Plunkett and Barr, 1956) that the patients with this condition are chromatin-positive (i.e. have cells containing a sex chromatin body)

and in 1959 Jacobs and Strong demonstrated that the karyotype was 47,XXY.

In retrospect it has turned out that not all of the original group of patients described by Klinefelter *et al.* were chromatin positive, and that those who were not showed a different testicular histology from those who were. And since 1959 many cases have been described as having Klinefelter's syndrome where the karyotype was not a pure 47,XXY, but a mosaic combining this with other cell lines (such as 46,XY/47,XXY, or 47,XXY/48,XXXY), or sometimes a still greater number of X chromosomes (such as 48,XXXY, or 49,XXXXY). Since these are all different genetic combinations, it seems best to start by describing the pure 47,XXY syndrome and to consider its variants later.

1. Incidence of the 47,XXY Syndrome

Figures for the incidence of this syndrome in the population are based on studies of the incidence of chromatin-positive males. It must be remembered that this group will include all the Klinefelter variants described above, and also the rather rare group of 46,XX phenotypic males. However the great majority of chromatin-positive males have the straight 47,XXY karyotype.

Extended series of sex chromatin studies on consecutive births have now been carried out, and Court Brown and Smith (1969) reported that in 41,000 consecutive births the proportion of chromatin-positive males was 1.7/1000. This is a slightly lower figure than that which has previously been accepted (on a smaller series), which was about 2/1000 (Court Brown, 1967). In this original series (Maclean *et al.*, 1964) 21 chromatin-positive males were found in 10,725 births, who included 12 who were 47,XXY, three who were 46,XY/47,XXY and one who was 48,XXYY. Clearly the condition is a relatively common one, with an incidence closely similar to that of Down's syndrome (*q.v.*), and since it is usually compatible, so far as is known, with a normal life span, it is a condition of considerable medical importance; there might be some scores of individuals with the syndrome to be found in the average Cup Final crowd.

2. Characteristics of the 47,XXY Syndrome

The syndrome is marked in general by the slightness of the phenotypic abnormalities. There are only two features which may have serious consequences for the subject. The first of these is seminiferous tubule dysgenesis. The testes of the adult male are extremely small (perhaps not more 1 inch in maximum diameter)

and on histological section they show small, hyalinized seminiferous tubules which may be composed entirely of Sertoli (supporting) cells and in which spermatogenesis is absent or slight. Leydig cells, by contrast, are prominent. This results in infertility. The other serious possible consequence of the 47,XXY karyotype is mental retardation. However this is not invariable, or even usual. Court Brown and Smith (1969) reported that the incidence of chromatin-positive males amongst 13,000 mental defectives examined was 9.4/1000. In comparison with the incidence of 1.7/1000 chromatin-positive individuals found at birth this figure certainly indicates an increased liability to mental retardation amongst chromatin-positive males. However there is not yet any satisfactory breakdown of this mentally defective group to show what proportion are 47,XXY and what proportion are mosaics and poly-X,Y, syndromes. There is no doubt that many, perhaps most males with the 47,XXY syndrome live normal lives, and are not very conscious that they are different from other men.

Apart from the two serious effects of the karyotype which have been mentioned, there are a number of other phenotypic effects which are commonly seen and which mostly result from the intermediacy of sex conferred by the karyotype. Most of these are variable in expression. They are gynaecomastia (enlarged breasts), female distribution of abdominal and facial hair, small phallus (the almost invariable presence of micro-orchidism has already been mentioned), eunuchoidism (i.e. a tendency to tallness and obesity combined with the underdeveloped secondary sexual characteristics already described), and oligospermy or azoospermy. Since most of these characteristics are only prominent after puberty it is not surprising that little is known of the physical characteristics before puberty; presumably the abnormalities are so slight that they are seldom detected (unless mental retardation is a feature).

Persons with the 47,XXY karyotype may present in various ways. They may be detected because of mental subnormality, or psychiatric disorder (a tendency to schizophrenia has been described). They may appear at infertility clinics, because of a sterile marriage; Ferguson-Smith *et al.* (1957) found that 3% of presenting males at an infertility clinic, and 11% of those with azoospermia, were chromatin-positive. They may turn up at endocrine clinics; they may present because of obesity, or complaining of the breast enlargement. Presumably a fair proportion never present at all. Court Brown (1967) has discussed this question in more detail, and gives some typical case histories.

It should be mentioned here that subjects with the 46,XY/47,XXY karyotype can show normal fertility in combination with many of the phenotypic features of the pure 47,XXY syndrome (Court Brown, 1967; Yunis, 1966). When it is borne in mind that mosaicism may often go undetected, it is likely that such cases account for some confusion in the literature as regards fertility in the 47,XXY syndrome. It is doubtful whether the syndrome in pure form is ever compatible with fertility, although it must be admitted that a number of 47,XXY individuals have been described who are fertile, and in whom no other cell line can be found in the most thorough investigations (Foss and Lewis, 1971). However the possibility of concealed mosaicism can never be excluded.

3. Genesis of the 47,XXY Syndrome

The syndrome must arise as a result of a non-disjunctional event taking place either in one of the parents, or in early development of the individuals themselves. Study of haemoglobin Xg can shed light on the origin of the error. This is (as mentioned above) an X-linked dominant gene which is extremely common, and can in suitable cases indicate the origin of the additional X chromosome. Race and Sanger (1969) have studied the distribution of the Xg gene in 47,XXY individuals, and find that it departs very significantly from that in normal males. This indicates that post-zygotic errors play little part in the genesis of the syndrome, and that most mosaics such as the 46,XY/47,XXY mosaics must arise as a consequence of non-disjunction or loss of a chromosome in an individual who has the 47,XXY constitution.

In about one-ninth of instances the Xg groups of the parents are such that it is possible to say whether the extra X chromosome is of maternal or paternal origin. In a fairly small series, Race and Sanger (1969) found that in about 40% the extra X was paternal ($47,X^{M}X^{P}Y$) and in the remaining 60% it was maternal. Of these five-sixths arose as a result of non-disjunction before or at the first meiotic division ($47,X^{M1}X^{M2}Y$) and one-sixth as a result of non-disjunction at the second meiotic division ($47,X^{M1}X^{M1}Y$ or $X^{M2}X^{M2}Y$).

Court Brown *et al.* (1969) have studied the age distribution of mothers of 47,XXY males and find that the mean maternal age (for a series of 163 males) is 32.4, which is significantly raised in comparison with the average maternal age for all births (28.3). In Race and Sanger's series of 18 cases in which the origin of the non-disjunction could be traced to father or mother, the mean age of

13 mothers in whom non-disjunction occurred was 32.1, and the mean age of five mothers when the non-disjunction took place in the father was 28.5. The difference was not statistically significant, but suggestive that the paternal errors were age-independent.

These findings are in line with the general finding that non-disjunctional errors of all sorts are partly age-dependent in mothers, but are age-independent in fathers of trisomic children. This subject is pursued at greater length in the section on Down's syndrome (Chapter 11) since more is known of it in this context owing to the extensive work of Penrose and others; and it will not be further discussed here. However it may be mentioned that Court Brown *et al.* estimated that in their series of 163 47,XXY males, 57% were of the sort arising independently of maternal age (Penrose class A) and 43% were maternal age-dependent. This must be regarded as a very rough estimate, but may be compared with Penrose's estimates of 33% Class A and 67% Class B in Down's syndrome (Chapter 11). In other words, although the incidence of the 47,XXY syndrome is about the same as that of Down's syndrome, the proportion of cases which arises as a consequence of non-disjunction related to maternal ageing is considerably lower.

Of course, to say that the 47,XXY syndrome is due to non-disjunction is not to give its aetiology—merely the mechanism. The aetiology of non-disjunctional errors will also be discussed later in relation to Down's syndrome, but it can be said here that little is known of it.

B. The 47,XXX Syndrome

This karyotype was first described by Jacobs *et al.* in 1959, and the name "super-female" (which has not stuck) was suggested. Up to 1963, 38 cases had been described (Day *et al.*, 1964). Subjects have two sex chromatin bodies visible in a proportion of cells from the buccal mucosa and elsewhere (see Chapter 7).

1. Incidence of the 47,XXX Syndrome

Maclean *et al.* (1964) reported nine certain and three probable cases in a series of 10,000 female neonates (the three probables being on the basis of sex chromatin studies without chromosome analysis), giving an estimated incidence of 1.2/1000 at birth. Of females institutionalized for mental defect rather more than 4/1000 have the 47,XXX karyotype, and this is significantly different from what might be expected from the birth incidence, indicating a tendency to mental defect. Hardly anything has been published on the incidence

in the "normal" adult population since nearly all the ascertainment of cases has been through the screening of inmates of institutions.

2. Features of the 47,XXX Syndrome

The syndrome is phenotypically inconstant (Yunis, 1966), but as in the 47,XXY syndrome, when abnormalities are found, they mostly involve either the brain or the reproductive system. Many subjects are apparently perfectly normal women, are fertile, and produce children. It might be expected that where children are produced, inevitable non-disjunction would lead to 50% of either 47,XXX or 47,XXY offspring but rather surprisingly no single instance of a child of a 47,XXX woman showing sex chromosome aneuploidy has yet been described. This suggests that there must be selection against any 22,XX gametes which are produced, which is not easy to explain in relation to the fact that such gametes are not selected against in this way when they arise as a result of accidental non-disjunction in normal women, when they can be fertilized and give rise to 47,XXY (and probably 47,XXX) offspring.

There is a tendency, as noted above, to mental deficiency, as in the 47,XXY syndrome. Court Brown (1967) comments that it is generally accepted that with an extra X chromosome in the complement the intelligence quotient (IQ) is reduced below what it would have been if the subject had had a normal sex chromosome complement. This may manifest itself in a tendency for the subject to drop in the social scale, sometimes through marriage, in relation to the karyotypically normal members of their family.

There is also a tendency to sexual abnormalities. Underdeveloped secondary sexual characteristics, amenorrhoea, sterility, menstrual disorders and deficient ovarian follicles in the ovaries have all been reported; but since population studies on adults have not yet been carried out on a large enough scale, it is not possible to say anything about the frequency of these conditions. It may be that failure of dosage compensation in the gonad (see Chapter 7), where the additional X chromosome may not be inactivated, is the reason for these abnormalities. It is even possible that all the fertile women reported might be hidden XX/XXX (or similar) mosaics where the XX line is only present in the gonads. But more information is needed on this subject.

3. Genesis of the 47,XXX Syndrome

Court Brown *et al.* (1969) report that the mean maternal age for mothers of 47,XXX females is (as for the 47,XXY males) significantly higher than that of the control population. Xg studies (Race

and Sanger, 1969) do not reveal the origin of the additional X chromosome in a series of 26 cases, since all but one were of the wrong constitution to shed direct light on the problem; but they were compatible with non-disjunction in the mother being the cause. The raised average maternal age, taken in conjunction with what is known of non-disjunction in other conditions, suggests that maternal age-dependence is one causal factor.

C. The 45,X Syndrome

This syndrome is commonly called Turner's syndrome. Turner (1938) first described a syndrome comprising infantilism, congenital webbed neck, and cubitus valgus (the angle between the humerus and the axis of radius and ulna, the "carrying angle", is increased). As with Klinefelter's syndrome, so with Turner's, the group of individuals who show some or all of these anomalies have turned out to have varying chromosome constitutions; but those with the 45,X karyotype show the main features of the syndrome with greater constancy than others, and therefore it seems best to start by considering the pure 45,X phenotype, and to consider related ones later.

Polani *et al.* and Wilkins *et al.* (1954) demonstrated that most cases of Turner's syndrome are chromatin-negative, and C. E. Ford *et al.* (1959) were the first to describe the 45,X karyotype in a patient with Turner's syndrome.

1. Incidence of the 45,X Syndrome

The incidence of chromatin-negative females at birth is about 0.4/1000 (Maclean *et al.*, 1964) and the majority of these are examples of the pure 45,X syndrome, though this figure will include the other karyotypes which can give a chromatin-negative female (see later). The incidence is lower than might be expected (if the condition arises as a result of a non-disjunctional event giving rise to monosomic and trisomic gametes, it might *a priori* be expected to arise with the same frequency as the 47,XXX syndrome). This is explained by the high frequency of the 45,X karyotype in spontaneous abortions; it has been estimated that 98% of all 45,X embryos are aborted (Chapter 9).

2. Features of the 45,X Syndrome

Virtually every anomaly of the sex chromosomes affects the brain and the reproductive system. The 45,X syndrome is unique not only

by being the only total monosomic condition normally compatible with life, but also because it does not normally result in mental defect. Individuals with the syndrome are not found more frequently in institutions than the incidence at birth might suggest (as is the case for the other syndromes described so far). This is surprising, because it is clear that the karyotype is associated with widespread physical abnormalities, including dermatoglyphic abnormalities; and not only are the skin and the nervous system usually linked—an abnormality in one implies an abnormality in the other (Chapter 12), but the brain is usually the most sensitive index of any condition producing developmental abnormalities. Some clinicians do believe that mental retardation is an occasional accompaniment of the syndrome (Valentine, 1969); but it is certainly not a striking feature of the typical case.

The most constant feature of the condition is ovarian dysgenesis. A "streak gonad" is usually found; small strands of ovarian tissue, on the back of the broad ligament, with elements of stroma but with a complete absence of germ cells and a virtual absence of endocrine tissue. As a consequence there is amenorrhea and failure of development of secondary sexual characters, with total infertility. This is the primary and most important consequence of the 45,X genotype.

However there are other and widespread physical abnormalities. Short stature (under 5 ft) is usual, and is not merely due to the endocrine deficiency. A short neck with webbing of the skin on either side is common, and this may be detected even at birth by the presence of looseness of the skin of the neck below the ears.

Cubitus valgus has already been mentioned. This is merely one aspect of widespread skeletal growth anomalies, which are manifested *in toto* in the short stature, and locally in various regions; deformity of the metacarpals is common; in the head a high arched palate, receding chin and malocclusion are found. General osteoporosis is often seen on X-ray. There is a delay in epiphysial union (due to the endocrine deficiency). The cardiovascular system often shows anomalies, in particular coarctation of the aorta, which is present in as many as a quarter of cases. At birth the 45,X infant is usually small (one-third weigh under 2500 g) and may have a soft, pitting oedema of hands and feet which can persist for weeks or months (Valentine, 1969). The chest is broad and shield-like, and the nipples are placed widely apart.

In the skin, apart from the abnormal dermatoglyphics (Chapter 12) pigmented naevi are often found; and in the adult there is a tendency to the formation of keloid scars after injury.

In short, there is evidence that many body tissues are abnormal (although, strangely, not the nervous system) and that this abnormality has been present from the inception of development.

3. Genesis of the 45,X Syndrome

Unlike most sex chromosome anomalies, the 45,X syndrome does not appear to be associated with a raised maternal age. Mean maternal age was found to be 27.6 (Court Brown *et al.*, 1969) which is well in the normal range. Race and Sanger (1969) estimated on the basis of Hb Xg studies, that the X chromosome was maternal in about 75% of cases and paternal in 25%. The Xg distribution was found to be of the male pattern, which is what is to be expected when only a single functional X chromosome is present. There are four ways in whcih an X chromosome could be lost (Court Brown *et al.*, 1969); it might be lost during gametogenesis of either ovum or sperm; it might be lost as a consequence of a post-zygotic error (with possible total loss of the normal cell line); or it might be lost between the initiation of fertilization and the fusion of the pronuclei. While the parental X distribution makes the first of these possibilities unlikely, there is not much evidence about the others; though the discovery by Sumner *et al.* (1971b) that a proportion of sperm (1.26%) from normal men appear to be disomic for the Y chromosome implies that a proportion of monosomic sperm is also a possibility (see Chapter 3).

4. Karotypic Variants of Turner's Syndrome

Since light is shed on the genesis of the various physical anomalies in Turner's syndrome by the fact that other karyotypes than the pure 45,X karyotype can be associated with some or all of the physical abnormalities of Turner's syndrome, it is pertinent to consider these karyotypic variants at this stage, before discussing the condition further.

The following karyotypes have been found in phenotypic females showing features of Turner's syndrome or the related syndrome of *pure gonadal dysgenesis:*

XO	XX	XXq–
XO/XX	XY	XXr
XO/XXX	XXpi	XO/XXpi
XO/XY	XXqi	XO/XXqi
XO/XYY	XXp–	XO/XXp–q–

This does not exhaust the possible variants, but gives an idea of the range of karyotypes found in these conditions.

Broadly speaking they may be classified into one of three groups; Turner's syndrome with mosaic karyotype; with normal male or female karyotype; or with a structurally abnormal sex chromosome.

(a) Turner's syndrome associated with a mosaic karyotype. The commonest mosaics found are XO/XX and XO/XY, and these are the only ones which will be discussed here, though a number of more exotic mosaics, sometimes with three cell lines, have been described (see under sex chromosome mosaicism).

(i) XO/XX mosaics. Like all mosaics, the eventual phenotype depends very much on the relative proportions of cells of the two lines, either in the body as a whole, or in individual tissues. Accordingly, any phenotype may be found in a spectrum between normal female and full-blown Turner phenotype. Ascertainment of cases of XO/XX mosaicism has usually taken place as a result of discrepancy between sex chromatin pattern and karyotype. It must always be remembered that any individual tissue may consist purely or almost purely of one of the two cell lines, and hence it is always possible that any apparently chromatin-negative female with the stigmata of Turner's syndrome may not be totally monosomic; nor need the presence of chromatin-positive cells in some body tissues rule out the possibility of an ovarian dysgenesis.

(ii) XO/XY mosaicism. The same rules apply to this type of mosaic. Since there is a possible spectrum of phenotypes ranging from normal male to Turner-type female, it is not surprising that cases of XO/XY mosaicism have been described as having perhaps the most variable phenotype of any regularly-occuring aneuploidy. This karyotype has been found in phenotypic females with gonadal dysgenesis, phenotypic males, or in intersexes with ambiguous genitalia (who may be considered as a variety of male pseudohermaphrodite).

(b) Turner's syndrome associated with 46,XX or XY karyotype. These may be divided into three groups, the first two of which do not show all the features of Turner's syndrome (and should not perhaps be considered under this heading at all, but can be discussed at this point because of parallelisms in the phenotype).

(i) The first group consists of 46,XX females who have normal ovarian function and secondary sexual characteristics, but show some physical stigmata, such as a webbing of the neck, resembling those of Turner's syndrome (Polani, 1962). Such cases may represent undetected mosaics or may be causally unrelated.

(ii) The second group comprises cases of *pure gonadal dysgenesis.* These have sexual infantilism, amenorrhoea and streak gonads

comparable to Turner's syndrome, but lack the other physical stigmata (except for cubitus valgus, which is often found). They are usually tall, eunuchoid in appearance and body proportions, and with either a 46,XX or XY karyotype. Since the sexually underdeveloped human being characteristically has a female phenotype of this sort, such cases may be explained on the basis of early failure of the gonad to develop and differentiate, possibly due to failure of the germ cells to migrate into the embryonic gonad, or to settle and survive there on arrival (see Chapter 3). This but perhaps only this, is the common feature which they share with classical Turner's syndrome.

Such cases may be familial. Chemke *et al.* (1970) described an interesting sibship in which two phenotypic females with gonadal dysgenesis and a phenotypic male with dysgenetic pseudohermaphroditism all had a 46,XY karyotype (there were also two normal sisters and a normal brother).

(iii) The third group do have both somatic and gonadal abnormalities of Turner's syndrome, and are most-easily explained as undetected XO/XX or XO/XY mosaics.

(c) Turner's syndrome associated with a structural abnormality of the sex chromosomes. Individuals with some or all of the phenotypic features of Turner's syndrome have been described who have the following sex chromosome anomalies; isochromosomes for the long or short arms of the X chromosome, deletions of either arm of the X, ring X chromosomes and abnormal Y chromosomes. Very small abnormal chromosomes of unknown origin have also been described.

(i) X chromosomes. Isochromosomes for the long arm of the X have been described; one X chromosome is missing, and an additional chromosome is found which in size and morphology resembles No. 3. Caspersson *et al.* (1970) have shown by fluorescent staining that such chromosomes are true isochromosomes displaying symmetrical bands of fluorescence similar to those in the long arm of the X. The sex chromatin body is found to be abnormally large in all cells in which it can be seen (Taft *et al.*, 1965) which fits with the observation that when a structurally-abnormal X chromosome is present it is always late-labelling and the presumed inactive member of the pair (Gianelli, 1963; Rowley *et al.*, 1964, and see Chapter 7). The drumsticks on the nuclei of the polymorphonuclear leucocytes are also large. Cases show all the signs of full-blown Turner's syndrome, including short stature.

Isochromosomes for the short arm of the X have also been

described, and Caspersson *et al.* (1970) have verified their nature by fluorescent staining. One of the two Xs is replaced by a slightly shorter and completely metacentric chromosome. The sex chromatin body is small, and may be found with a lower frequency than usual. Drumsticks may be absent. The phenotype resembles the Turner phenotype, except that stature is normal. There are however some somatic stigmata; a short fourth metacarpal, and pigmented naevi, may be found (Jacobs, 1969). Patients with these isochromosomes are often mosaics (see later).

(ii) Deleted X chromosomes. Both 46,XXp- and XXq- have been described. Deletions of the short arm give all the phenotypic signs of full-blown Turner's syndrome, but a sex chromatin body is present. Deletions of the long arm are associated with a phenotype rather similar to that for isochromosomes for the short arm; streak gonad and sexual immaturity, but normal stature, and not even the limited somatic abnormalities found in XXpi. Sex chromatin bodies are found, but are abnormally small and found with a lower than normal frequency.

It has been deduced from the phenotypic findings in individuals with X chromosome deletions that genes on both arms of the X chromosome must be present for normal ovarian development to take place; but that the genes which prevent short stature (and webbing of the neck) are on the short arm. Two short arms of the X chromosome must be present for stature to be normal (Jacobs, 1969). Other explanations are, however, possible (Hecht *et al.*, 1970), although this one certainly seems the simplest.

(iii) Ring X chromosome (XXr). This has been described in association with gonadal dysgenesis (Lindsten and Tillinger, 1962; Yunis, 1966). Presumably gonadal dysgenesis in such cases would be determined by the extent of the deletion of both arms which is present. Caspersson *et al.* (1970) were unable to detect the extent or the site of the presumed deletion by fluorescence studies on one such case.

(iv) Abnormal Y chromosome. Structurally abnormal Y chromosomes are rare but Jacobs and Ross (1966) described two cases thought to have a possible isochromosome for the long arm of the Y. Both had the phenotype of an immature female. One had a streak gonad; but neither was of short stature. One had a short neck and the other a deep voice, but there were no other signs suggestive of Turner's syndrome (or of masculinization). These cases suggest that it is possible that the genes controlling testicular development are on

the short arm of the Y, while genes preventing the development of the somatic features of 45,X are on the long arm, but the conclusion is very tentative.

(v) Presence of a small, abnormal chromosome of unknown origin. A few cases have been described in which the Turner phenotype has been associated with a 46,XX? or Y? karyotype. The small abnormal chromosome is either a small centric fragment or a ring, and because of the small size autoradiography has not been of help in distinguishing between a deleted X and a deleted Y; the latter possibility can only be suspected if there are some masculine features present. Cohen *et al.* (1967) studied four patients of this sort autoradiographically; each was a 45,X/46,X plus minute chromosome mosaic. Cohen *et al.* suggested that the XO cell line appeared because the X chromosome was vulnerable to loss of its late-replicating, heterochromatic region, and that cases which were apparently XO might not always have been monosomic, but might have once possessed a small ring X chromosome or a centric fragment which was subsequently lost.

5. Incidence of Structurally Abnormal Sex Chromosomes in Turner's Syndrome

Structural abnormalities of the sex chromosomes appear to be rather rare in the population at large. Jacobs (1969) reported that only two examples (one a deleted X and the other a ring X) were found in 64 cultures performed on a group of 108 newborn babies found to have abnormalities of the sex chromosomes (from a sample of 82,000). However they are more common in Turner's syndrome than might be expected from this; C. E. Ford (1969) reported that in 28 of a series of 163 cases of Turner's syndrome there was a cell line containing a structurally abnormal sex chromosome. Lindsten (1963) found in a series of 56 cases of Turner's syndrome that two had isochromosomes of X, and no less than nine further cases were XO/XXi mosaics. Where a structurally abnormal X chromosome has been found, there has usually also been found another, 45,X cell line; this applies to deletions and isochromosomes, with the exception of the isochromosome for the long arm, 46,XXqi. This perhaps relates to the known tendency for even normal X chromosomes to be lost with advancing age; an abnormal X (which is usually inactive and heterochromatinized) may show a stronger tendency to be shed. C. E. Ford (1969) prefers a slightly different explanation. He considers that the frequency of mosaicism in Turner's syndrome (see above) combined with the fact that there is seldom a normal XX line

in mosaics, suggests that a single event often accounts for both lines; this certainly affords an easy explanation for XO/XXi mosaics.

D. The 47,XYY Syndrome

The first individual with this karyotype was described by Hauschka *et al.* (1962), and was both developmentally normal and fertile (an individual with the 48,XXYY karyotype had previously been described by Muldal and Ockey in 1960). The first description of the finding of a number of males with two Y chromosomes was published by Casey *et al.* (1966). They studied 21 chromatin-positive males ascertained by sex chromatin examination of 942 inmates of two State Hospitals for patients requiring special security because of persistently violent or aggressive behaviour (Rampton and Moss Side). The majority of the 21 chromatin-positive males were examples of Klinefelter's syndrome, or Klinefelter mosaics, but no less than seven had two Y chromosomes, five were 48,XXYY and two 47,XYY/48,XXYY. This finding suggested a causal connection between the possession of two Y chromosomes and the behavioural pattern which necessitated care in such institutions, and Jacobs *et al.* (1965) tested the hypothesis by karyotyping 197 of a total of 303 patients, considered to be mentally subnormal, and confined in a similar State Hospital in Scotland (Carstairs). Nine of these had sex chromosome aneuploidies, including no less than seven who were 47,XYY and one 48,XXYY (the remaining one being 47,XXY). When a further 117 patients were examined out of 139 in a wing of the hospital for psychotic patients two more 47,XYY males were found. Another significant observation was made on the XYY males discovered; they were characteristically tall. Their mean height was greater than that of 46,XY males in the same hospital, and eight out of nine of them were over 6 ft in height.

A control experiment to the above was carried out by Close *et al.* (1968). They studied sex chromatin in mentally subnormal males in ordinary hospitals for the mentally subnormal. Eleven of 917 males were found to be chromatin-positive. Interestingly it was found that four of these 11 were found in a group of 82 men admitted under the Mental Health Act, 1949 (4.7%), while only 0.9% of those admitted informally were chromatin-positive (7/824). It was not possible to karyotype such a large number, but chromosome studies were undertaken on 68 of the tallest. Nineteen of these were over 6 ft in height, and this group contained two 47,XYY and one 48,XXYY individuals (together with 46,XY and one 47,XXY). The

remaining 49, who were between 5 ft 10 in and 6 ft in height, contained a further two 47,XXY individuals.

These findings clearly suggested a possible association between the posession of two Y chromosomes, tallness, and antisocial behaviour.

1. Incidence of the 47,XYY Syndrome

The fact that the 47,XYY syndrome was not described until 1962, and that few cases were reported until after publication of the work outlined above, suggested at first that the condition was rare. Price (1969) suggested that probably not more than 1/2000 of the general population were XYY. Later reports (Ratcliffe *et al.*, 1970), give a best estimate of 1.7/1000 males. However it has not been possible until 1970 to screen a random series of newborn children in the way that has been possible for the X chromosome using sex chromatin. The discovery by Pearson *et al.* (1970) of Y-fluorescence in the interphase nucleus has now made this possible, and an estimate based on a larger series will no doubt soon be available. If the incidence is as high as 1.7/1000 males (and there are no reports that the genotype is associated with any abnormal mortality rate) it is likely that the majority of individuals with this genotype are virtually indistinguishable from normal males.

2. Features of the 47,XYY Syndrome

The main features of the syndrome have already been outlined; there is a tendency to tall stature, to mental defect, and antisocial behaviour. What is not yet known is how frequent are these traits in the whole group of XYY individuals; presumably there are many 47,XYY males who do not differ significantly from the population at large in either height, mental capacity, or emotional stability; whether they differ in these respects from the individuals they would have been if they had not possessed two Y chromosomes is another matter on which there is as yet inadequate evidence: analogy with the 47,XXX and XXY syndromes suggests that they may.

Apart from these abnormalities, no physical abnormalities have been described as of regular occurrence in 47,XYY males. There is throughout, however, a suggestion that their troubles stem from an overdose of maleness.

Parents who have children of both sexes can hardly fail to notice that there are differences of temperament which exist at the earliest age. The male child is more aggressive, and has different interests from the female. In later childhood this expresses itself as an interest on the one hand in games involving fighting, competition and

machinery, and on the other hand, interest in dolls, babies and domestic affairs. It is not difficult to imagine the effects of a double dose of male psychological attributes. If this is combined with low intelligence it is not surprising that it results in antisocial behaviour. However if combined with normal or high intelligence a true "supermale" might result. But, as with the 45,X syndrome, although the intellect does not necessarily seem to be impaired, the fact that the dermatoglyphic pattern may be different from the normal implies that the nervous system may be different also, though this may not be manifest.

Confirmation that the behaviour of 47,XYY patients in institutions is due to their extra Y chromosome comes from a study of their behaviour and history (Price and Whatmore, 1967). As compared to a control group of criminals there are three distinctive findings. Their delinquency started at an earlier age; their siblings had very few convictions in comparison to the siblings of the control group of criminals; and they committed fewer crimes against the person and more against property.

Hunter (1966) suggests that the reason these 47,XYY individuals are concentrated in State hospitals is the frightening picture presented by the combination of great height and build, combined with low intellect and a criminal history. But there is probably more to it than this.

As mentioned before, the 47,XYY individual may be (and probably usually is) normally fertile. It might be expected that inevitable non-disjunction during meiosis might lead to 50% of 47,XYY offspring. However although at least two instances have been reported of a 47,XYY father and son, the impression is that this is unusual, and that there is probably selection against the 24,YY gamete (Izona-Neva *et al.*, 1966; Hauschka *et al.*, 1962).

3. *Genesis of the 47,XYY Syndrome*

Mean maternal age does not appear to be raised in the 47,XYY syndrome. The mean age of 55 mothers of cases was 27.8 (Court Brown *et al.*, 1969). Of course, since an abnormal meiotic event must occur in the father, there is no direct reason why maternal age should be raised; but if the average paternal age was raised, a secondary effect might be expected in maternal age. The average paternal age in this series was 2.3 years more than the maternal, which is within the normal range.

The meiotic accident which gives rise to a 24,YY spermatozoon must of course be non-disjunction at the second meiotic division.

Sumner *et al.* (1971a) have found, from fluorescent staining of spermatozoa, that about 1.25% of them (in a series containing both normal and infertile males) contained two fluorescent bodies which probably represented the Y chromosomes. If these were all as viable as euploid spermatozoa, and the primary sex ratio taken as unity, it would mean that about 2.5% of males conceived would have the 47,XYY genotype. Since only 0.17% of males appear to have this genotype at birth, on present estimates (*vide supra*), and there is no evidence from studies on abortuses that 47,XYY individuals are frequently aborted (though it is possible that some abortuses recorded as trisomy for a G chromosome are in fact 47,XYY) it would seem that there is very considerable selection against the 24,YY gamete, which only hits the target successfully one time in 15 or so.

The finding of a high number of 24,YY sperm in normal males is in interesting contrast to the observations of Thompson *et al.* (1967) who studied meiosis in a 47,XYY male. They found no single spermatocyte of 155 analysed which clearly contained two Y chromosomes (they did not of course have the benefit of fluorescent techniques), and postulated a selection towards euploidy at meiotic or even premeiotic level. It is likely that fluorescence will shortly shed much more light on this topic.

Xg studies will, of course, shed no further light on the origin of the 47,XYY syndrome. It can however do so with the related 48,XXYY syndrome. Race and Sanger (1969) report two cases out of a series of ten where this is so, and in both of them the evidence indicated that non-disjunction occurred at both the first and second meiotic divisions in the father (see also De la Chapelle *et al.*, 1964).

The findings of Sumner *et al.* (1971a) of 1.25% of YY sperm implies that there should be a corresponding number of 22,O sperm, which might, if as viable as normal sperm, give rise to 1.25% of 45,XO conceptuses. This is over 60 times the number of 45,X individuals found at birth, but it will be remembered that it has been estimated that 98% of 45,X conceptuses may be aborted. If the number of XO gametes arising from non-disjunction during oogenesis is taken into account, it seems quite possible that the 22,O sperm is in every way viable as the euploid sperm, and is not selected against.

The XYY phenotype may arise as a result of a structurally abnormal Y chromosome. El-Alfi (1970) has reported a very interesting family consisting of a father and three sons, all of whom show behavioural disorder, skeletal overgrowth, large external genitalia and an extra electrophoretic protein band following that for

β-globulin. All four had an extremely long Y chromosome, interpreted as a duplication of a large part of the long arm.

E. Poly-X and Y Syndromes

Individuals with larger numbers of X and/or Y chromosomes than in the basic 47,XXX, XXY or XYY syndromes show, on the whole, similar features to the basic syndromes which they resemble, but in a more pronounced fashion. These conditions are all rather rare, so it is unwise to generalize about them until a statistical picture can be built up; there is a general impression from recorded cases that the greater the number of sex chromosomes, the greater the likelihood (or the degree) of mental subnormality and of developmental anomalies generally. But it must always be remembered that as is usual in sex chromosome anomalies, most cases are ascertained because they are abnormal, and that individuals who are completely or relatively normal are unlikely to be discovered. The rarer the condition, the more likely is this to be so.

Many individuals with these abnormalities are mosaics, as is the case for sex chromosome anomalies generally. Mosaicism will be discussed in the next section, and only pure forms of these syndromes considered here.

1. Incidence of Poly-X and Y Syndromes

These syndromes are all very rare. In a study by Maclean *et al.* of a series of 10,000 female and 10,725 male newborn children, no female with more than three nor male with more than two X chromosomes was found. It would probably be necessary to do a series of 100,000 to get any true picture of the incidence.

2. Features of Poly-X and Y Syndromes

(a) Poly-X syndromes. The 48,XXXX syndrome is so rare that the total number of cases reported scarcely reaches double figures. The usual description is of a female who is almost normal physically, but who is usually severely mentally defective. At least one case has been described however with an I.Q. of 75 (Telfer *et al.*, 1970), so mental defect is not invariably severe. There are dermatoglyphic abnormalities (low ridge-count, see Chapter 12). Most cases appear to be sexually normal, but there is no evidence as to whether they are fertile or not.

The 49,XXXXX syndrome is excessively rare, but has been reported on at least one occasion (Keseree and Woolley, 1963). Up

to four sex chromatin bodies were found in the buccal mucosa, and up to three drumsticks on the polymorphs.

(b) Poly-X Y syndromes. 48,XXXY and 49,XXXXY syndromes are both known. The 49,XXXXY syndrome seems to be the commoner of the two, judging by the number described in the literature (although this could be a false criterion; see above). Both syndromes show features of Klinefelter's syndrome in a more pronounced fashion. The additional X chromosome(s) seem to increase the degree of testicular hypoplasia and the degree of mental retardation. All reported cases seem to be mentally defective, most severely so.

In the 49,XXXXY syndrome there seem to be additional physical abnormalities. As well as severe mental defect (Alonsson *et al.*, 1966, report that the I.Q. range in most reported cases is between 20 and 60) and a more marked genital hypoplasia than is found in the 47,XXY syndrome (often including undescended testes) a rather regular anomaly seems to be proximal synostosis of the radius and ulna. Other physical anomalies which have been described in some cases include hypertelorism, epicanthic folds, palatal abnormalities and dermatoglyphic abnormalities including simian line (and clinodactyly).

The 48,XXYY syndrome is now also well known. Fifteen cases had been described up to 1965; after this date many more cases were uncovered in state hospitals for patients requiring special security (see above). As might be expected, these individuals show combined features of the 47,XXY and 47,XYY syndromes. Since both of these confer increased height on their possessors, it is not surprising that the 48,XXYY individuals are abnormally tall as a group. In the series uncovered by Casey *et al.* (1966), seven 48,XXYY individuals were on average about 5 cm taller than twelve 47,XYY individuals, and this was all accounted for by increased leg length. This is no doubt due to the increased growth potential conferred by the extra Y acting on the eunuchoid constitution (with late-closing epiphyses) of the XXY phenotype.

Again just as both 47,XXY and 47,XYY individuals have a tendency to mental defect and personality disorders, so it is found that 48,XXYY individuals suffer from these troubles in a more extreme way.

Individuals with larger numbers of X and Y chromosomes than this have been described, but are so rare that no generalizations can be made.

3. Genesis of Poly-X and Y Syndromes

Because of the rarity of these conditions there are not many informative cases. Race and Sanger (1969) found that in one case out of nine 48,XXXY, three of twenty-six 49,XXXXY and probably one of two 48,XXXX, all the X chromosomes were maternal, indicating that they had arisen through non-disjunction at both meiotic divisions of oogenesis. There were no cases in which paternal origin of extra X chromosomes could be proved. In two of ten 48,XXYY individuals studied, Xg evidence indicated that the origin was double non-disjunction in the fathers giving rise to 25,XYY sperm.

III. SYNDROMES WITH NORMAL SEX CHROMOSOMES AND ABNORMAL PHENOTYPE

A number of cases have been described in which a normal chromosome constitution is associated with apparently paradoxical phenotypic sex. An important group is that of 46,XY phenotypic females with testicular feminization. Much more rare are 46,XX males, and 46,XX Klinefelter's syndrome. Since this book is concerned with the chromosomes themselves rather than with sex determination, and the whole problem of human intersexes is a large and complex one, of which there are a number of good reviews, no more will be done here than to summarize the main findings in the groups mentioned above.

A. Testicular Feminization with 46,XY Karyotype

Affected males have a female phenotype, with female external genitalia, breast development, hair distribution, etc. There is a blind vagina with absent uterus, and testes are present but undescended (patients often present because of a presumed inguinal hernia). The phenotype is often voluptuously female, and the condition has been described in photographic models, airline hostesses and prostitutes (McKusick, 1966).

The condition may be familial, and although the mode of inheritance is uncertain (and may not be the same in all cases) it is usually considered to be due to an X-linked gene, possibly recessive but more probably dominant (Hamerton, 1969), although an autosomal gene cannot be ruled out in all cases. The gene is thought to act by making body tissues resistant to the action of testosterone.

McKusick (1966) has described an interesting case of a woman who was sister, mother and grandmother of affected males, and who showed asymmetry of development of secondary sexual characteristics; in particular the right breast was smaller than the left and there was no pubic hair to the right of the midline. McKusick considered that this was evidence for a sex-linked recessive gene, and the patchy changes might be due to Lyonization of the X chromosome concerned.

The condition is rare; the incidence has been estimated at 1/65,000 at birth.

B. 46,XX Male Syndrome (and 46,XX Klinefelter's Syndrome)

This is a very rare condition; affected individuals may have apparently normal male phenotype and libido—with testes (but will of course be sterile), or may have the Klinefelter phenotype. Boczkowski *et al.* (1969) and Lisker *et al.* (1970) have described phenotypically normal males with the condition; Boczkowski *et al.* found that their case had abnormal dermatoglyphics—a high ridge count, similar to that of Turner's syndrome, and an *atd* angle intermediate between the mean for normal males and females and that for Turner's syndrome (see Chapter 12). The testis contained Sertoli cells only.

At least seven cases have been described where the typical phenotype of Klinefelter's syndrome has been associated with a 46,XX karyotype (Hamerton, 1969), and no Y chromosome could be detected in any tissue examined.

Three hypotheses might explain either condition:

1. Concealed mosaicism. This clearly cannot be disproved, but has not been detected in the cases described.
2. The male-determining gene(s) on the Y chromosome have been transferred to an X. This again is hard to disprove. Philip *et al.* (1971) have shown that no fluorescent Y-body can be detected in the Boczkowski case (but there is no evidence that the male-determining genes lie in the fluorescent long arm of the Y).
3. The condition is due to the operation of an autosomal sex-reversing gene. Because of the rarity of the condition, there is no genetic evidence to support or disprove this hypothesis. However it is known from the example of the gene "polled" in the goat, that such mechanisms can exist. The "polled" recessive gene, when present in double dose, produces a female

goat which is a phenotypic male, with testicular development and normal male libido. After birth the testes contain no germ cells; it has been shown that these migrate to the testis early in embryonic life, but die and disappear during the prenatal period (Hamerton *et al.*, 1969).

IV. SEX CHROMOSOME MOSAICS AND CHIMAERAS

A. Definition

A *mosaic* may be defined as an individual or organism displaying genotypic or phenotypic variations from cell to cell within the same tissue or between tissues; in cytogenetics this refers in practice to chromosomal variation between different cells, all descended from a common zygote lineage.

A *chimaera* is an organism or individual which is not genetically uniform throughout; the cells are of more than one zygote lineage, because of mutation, anomalies of fertilization and early development, or grafting.

B. Mosaicism of the Sex Chromosomes

The subject has recently been reviewed by C. E. Ford (1969). Numerical mosaics for the sex chromosomes may be classified by the number of different cell lines present and the minimum number of mitotic events that must be postulated to account for them.

1. Possible Cell Lines Arising from One Non-disjunctional Event Involving the Sex Chromosomes in a Normal Man or Woman (given in Table 7).

Unless the non-disjunction occurs at the first cleavage division, three cell lines will be present in the body: the normal line, and hyper- and hypo-diploid lines arising as a consequence of the non-disjunction. All three lines may persist, or one of them may be lost, and this can give four possible mosaic combinations for each sex (ignoring the possibility that all cell lines except one are lost, in which case the individual is no longer a mosaic). All of the eight possible mosaics arising from one non-disjunction in normal men and women have been described (C. E. Ford, 1969). Estimates of their incidence are not available, but they are all rather rare. It is not possible to generalize about the phenotype since this will depend on the stage of development at which non-disjunction occurs, and on the proportions of the different cell lines present in the body as a whole and in particular tissues (especially the gonads). As a general rule the

Table 7

Sex chromosome mosaics

1. Possible mosaics arising from a single non-disjunctional event

Original sex chromosomes	Possible mosaics	Original sex chromosomes	Possible mosaics
XX	XO/XXX*	XY	XO/XYY*
	XO/XX/XXX		XO/XY/XYY
	XO/XX*		XO/XY
	XX/XXX		XY/XYY*
XXY	XO/XXXYY		XO/XXY
	XO/XXY/XXXYY		XXY/XXXYY
	XX/XXYY		XX/XXY
	XX/XXY/XXYY		XXY/XXYY
	XY/XXXY		XY/XXY
	XY/XXY/XXXY		XXY/XXXY
XXX	XO/XXXX		XO/XXX*
	XO/XXX/XXXXX		XXX/XXXXX
	XX/XXX/XXXX		XX/XXX*
	XX/XXXX		XXX/XXXX
XYY	XO/XYYYY		XO/XYY*
	XO/XYY/XYYYY		XYY/XYYYY
	XY/XYYY		XY/XYY*
	XY/XYY/XYYY		XYY/XYYY

2. Some mosaics which have been described. which must result from at least two non-disjunctional events

XO/XXXY	XX/XY/XYY
XO/XXY/XXYY	XX/XXY/XXYYY
XO/XX/XY	XYY/XXYY

Underlining indicates that the mosaic combination has been described in the literature.
* Indicates that the mosaicism can arise from more than one original sex chromosome complement.

phenotype relates to the cell line(s) which predominate (although the possibility must always be borne in mind that a cell line may have been predominant during some important stage of development and then either died out or become proportionately much reduced).

Little is known of changes in proportions of cells of different lines during the development of sex chromosome mosaics. However what

little is known of such changes in autosomal mosaics (Chapter 11) suggests that the relative numbers of cells of different cell lines do not remain constant during development, but may fluctuate considerably. Since every mosaic individual is unique in the site and time of the non-disjunctional event which has made them mosaic, there is little point in generalizing about what may happen. Only study of the phenotype, developmental history, and of the cell lines found in as many tissues as can be examined, will give guidance on the genetic composition of such individuals.

2. Mosaics Arising from One Non-disjunctional Event in Aneuploid Individuals

Since both sex chromosome mosaicism and aneuploidy are fairly rare only the commonest aneuploidies will be considered here—47,XXX and 47,XXY.

One non-disjunction in the 47,XXY syndrome can give 12 possible mosaic combinations (if it is assumed that one cell line may be lost), which are listed in Table 7. At least half of these have been described. Of course, some of them might have arisen in other ways. Thus the XX/XXY combination might arise by simple loss of the Y chromosome in one cell line.

One non-disjunction in the 47,XXX syndrome can give eight possible mosaic combinations (Table 7). Two of these (XO/XXX and XX/XXX) can also arise from non-disjunction in a 46,XX woman, and the latter eventuality is obviously more probable. Of the other combinations, the XXX/XXXX combination has been described; the author has not encountered descriptions of the others.

One non-disjunction in the 47,XYY syndrome can give eight possible mosaic combinations. Two of these can also arise as a result of one non-disjunction in a normal male (XO/XYY and XY/XYY). Examples of the others have not been yet encountered, but may well be discovered as a consequence of the development of the fluorescent Y-body technique.

3. Mosaics Arising from More Than One Non-disjunctional Event

The enumeration of possible mosaics arising from more than one non-disjunctional event in normal or aneuploid individuals has a certain mathematical fascination, but not much practical significance. It suffices to say here that a number of mosaics have been described which can only be explained on the basis of two such events. Among these are XO/XXXY, XO/XXY/XXYY, XO/XX/XY, XX/XY/XYY and XX/XXY/XXYYY (C. E. Ford, 1969).

C. Sex Chromosome Chimaeras

Chimaerism is excessively rare, but the possibility should always be borne in mind that an individual with more than one cell line may be a chimaera. The evidence for chimaerism must come from studies on blood groups and other genetic markers which show different cell lines which must have originated from more than one zygote or individual. C. E. Ford (1969) has described nine possible types of chimaera, of which six have been described in man; these are as follows:

1. Chimaerism Due to Two Separate Acts of Syngamy

1. Dispermy with suppression of second meiotic division. Not found.
2. Dispermy with fertilization of ovum nucleus and first polar body nucleus. Not found.
3. Dispermy with fertilization of ovum nucleus and second polar body nucleus. A case described by Zuelzer *et al.* (1964).
4. Dispermy with fertilization of two haploid nuclei, daughters of the ovum nucleus (Gartler *et al.*, 1962). Equal division of the primary oocyte to give two equal-sized daughter cells has been described in mice (Donahue, 1970).
5. Fusion of one daughter of the zygote nucleus with the nucleus of a fertilized second polar body (Schmid and Vischer, 1967).

2. Chimaerism Due to Contributions from Two Independent Zygotes

6. Early fusion of two separate embryos. Not found in man, but has been achieved experimentally in mammals (Tarkowski, 1961).
7. Placental cross-circulation between dizygotic twins, as found in the freemartin condition of cattle, where chimaerism is the rule (Woodruff *et al.*, 1962).
8. Maternal-fetal transplacental exchange (Taylor and Polani, 1965).
9. Artificial chimaerism (iatrogenic) due to transfusion or grafting (Naiman *et al.*, 1966).

It is sufficient in a book this size to draw attention to the possibility of chimaerism, and to discuss how the condition may arise, and the topic will not be further pursued.

V. STRUCTURAL ABNORMALITIES OF THE SEX CHROMOSOMES

Structural abnormalities of both the X and Y chromosomes have been described on a number of occasions. Because of the unique nature of the two sex chromosomes it is not always easy to define a structural alternation. For example, in the rather genetically inert Y chromosome, it is not easy to say at what precise point a short Y (which may be functionally normal) is deleted, or a long Y is duplicated; or how significant such deletions or duplications are if they involve heterochromatic areas of the chromosome which may consist of redundant or inert DNA.

Reciprocal translocations involving the X or Y seem to be very rare, although this may also be related to difficulty of detection. The new fluorescent techniques should enable Y/autosome translocations to be detected, if they occur with any frequency.

A. Structural Abnormalities of the X Chromosome

These have largely been considered under Turner's syndrome (*q.v.*), in which isochromosomes for both the short and long arms, deletions of either arm, and ring chromosomes, have been found, often in association with mosaicism. Indeed, the frequency of such mosaicism (the association of a 45,X line with a line containing an abnormal X chromosome) taken in conjunction with the rarity of structurally abnormal X chromosomes in the absence of Turner's syndrome, has suggested to C. E. Ford (1969), as already mentioned, that the mosaicism, abnormal X and Turner phenotype all arise as a consequence of a single event.

It has already been postulated (Jacobs, 1969), on the basis of the phenotypes of patients with isochromosomes and deletions of the X chromosome, that genes on both arms of the X chromosome must be present for normal ovarian development; whereas the genes which control stature, and webbing of the neck, are located on the short arm. For these two physical features to be normal, it is necessary that short arms from at least two X chromosomes be present.

B. Structural Abnormalities of the Y Chromosome

These are uncommon and are usually associated with infertility (Jacobs, 1969).

1. Structurally Abnormal (or Unusual) Y Without Genetic Effect

As previously mentioned, Y chromosomes have been described in families without genetic abnormality, which may be very long (> E18), long (> F20) or very short and metacentric (< ½G21), but which clearly carry all the genes necessary for normality, and not in excessive dose.

2. Duplication of the Y Chromosome

El-Alfi (1970) has described a case of a father and sons with a long Y chromosome and symptoms of the 47,XYY syndrome (see above). This case is at present unique in the literature, but shows that tandem duplication of the Y can occur.

3. Deletion of the Y Chromosome

Some of the cases of Turner's syndrome where there are 46 chromosomes which include one X and a small fragment may represent instances of a deleted Y; but the fragment is usually so small that its identification as either X or Y is inconclusive.

4. Y Isochromosomes

Probable isochromosomes of the Y have been described by Klevit *et al.* (1963) and Jacobs and Ross (1966). The two cases of Jacobs and Ross had the phenotype of an immature female. Taken in conjunction with the fact that a short metacentric Y is compatible with normal testicular development, it may be hypothesized that the genes responsible for normal testicular development lie close to the centromere on the short arm of the Y (Jacobs, 1969).

5. Dicentric Y Chromosomes

A number of cases have been described where a dicentric Y chromosome is present. These abnormal chromosomes may be due to a Y/Y translocation (as in the case of Yunis, 1965) or to loss of part of the short arm with sister chromatid reunion (as was considered to be the case by Jacobs in the example described by McIlree *et al.* (1966) and Boschetti *et al.* (1968). The case of McIlree *et al.* presented as a case of infertility and no second meiotic metaphases were found in the testis; univalent X and Y chromosomes were found at diakinesis in most first meiotic metaphases, which might have been related to the breakdown of meiosis. However, cases in which a dicentric Y has been passed from one generation to the next have been described by Starkman and Jaffé (1967) and by Sparkes *et al.* (1970).

By contrast, Angell *et al.* (1970) have described three cases of dicentric Y chromosomes associated with ovarian dygenesis in phenotypic females.

6. *Translocated Y Chromosomes*

Translocations involving the sex chromosomes are (as mentioned earlier) very rare. Apart from the dicentric Y interpreted by Yunis (1965) as a Y/Y translocation, the only example found by this author is the case of Federman *et al.* (1967) where an individual with mixed gonadal dysgenesis was considered to be a mosaic of 45,X cells, and cells with 46 chromosomes including a presumptive D/Y translocation.

Reitalu *et al.* (1970) have postulated that many cases of hypogonadism may be due to structural interchanges between the X and Y chromosomes, but the evidence for this is unconvincing.

§11§

Abnormalities of the Autosomes

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I. INTRODUCTION

An alteration in the genetic content of the autosomes, whether due to aneuploidy or to duplication or deficiency of part of a chromosome, leads almost inevitably to abnormality of the phenotype. This is in contrast to abnormalities of the sex chromosomes, where the genetic inertness of the Y chromosome and the inactivation of all X chromosomes except one means that excess or deficiency of genetic material may be consistent with a normal or nearly normal phenotype.

It might be thought that since somatic chromosomes exist in homologous pairs, the presence in a cell of one member of the pair would ensure that the necessary genes were present, and would be

compatible with life. But this is found to be not so; monosomy for an autosomal pair seems to be almost invariably lethal (and at a very early stage of development, since it is rare even in early abortuses), and is not found in the postnatal population, with the possible exception of monosomy of one of the group G autosomes (see later).

The only common and socio-economically important condition found in the postnatal population which is due to an autosomal anomaly is Down's syndrome (mongolism), which is associated with trisomy for the chromosome which is conventionally called No. 21, but which should really (on grounds of size) be called No. 22; that is, the smallest of all the autosomes. Down's syndrome, with an incidence at birth of about 1.5/1000, and which, despite a high mortality in childhood is compatible with survival to middle age, presents a major social and economic problem all over the world. Most other conditions associated with autosomal anomalities are either incompatible with survival beyond early childhood (e.g. the other known autosomal trisomies) or are rare and sporadic in incidence (e.g. the range of unrelated conditions associated with structural rearrangements of the autosomes). This is not to say that they do not represent economic problems, especially in advanced countries; an infant with the severe malformations associated with trisomy for chromosomes No. 13 or 18, which would probably perish soon after birth in a country without advanced medical facilities, may survive for an average of three months or so in an intensive care unit. In a country such as the U.S.A. this can be a major, and totally unrewarding, economic drain on the parents.

Since this book is primarily concerned with what chromosomes are and how they work, and with principles rather than details, it is not intended to make a catalogue of all the many conditions due to autosomal anomalies which have been described in the literature; but to confine description to those conditions which are of regular appearance, and then to consider in a general way the conditions which appear sporadically and which are inconstant in phenotypic manifestation.

II. CLINICAL CONDITIONS DUE TO ANEUPLOIDY OF THE AUTOSOMES

While a wide variety of autosomal aneuploidies have been found in aborted embryos (see Chapter 9) the only ones which are compatible with postnatal life are three autosomal trisomies (47,21+, 47,13+ and

47,18+) and the very rare cases of triploidy (69) and monosomy (45,G–), which are almost always mosaics with a normal cell line also present (except in one or two reported cases of monosomy G where no normal cell line has been demonstrated).

A. Down's Syndrome (Typically 47,XX or XY,G+)

1. Introduction

The syndrome has been authoritatively reviewed by Penrose and Smith (1966) and will therefore only be treated in outline here. The condition has been recognized for over 100 years, since it was first described by Langdon Down (1866) and has an enormous literature which it is impossible to cover adequately in a whole chapter, still less a subsection. Down called the condition "mongolian idiocy" and the name has stuck, though many other eponyms have been suggested; Kalmuk idiocy, unfinished child, ill-finished child, acromicria, generalized foetal dysplasia, peristatic amentia, and so on. Recently the name Down's syndrome (or disease, anomaly, etc.) has become generally accepted, and is to be preferred to the still popular and unsuppressable "mongolism" which was so long taught to all medical students. The term "mongolism" is unsatisfactory not merely because the resemblance of sufferers to members of Oriental races is superficial and misleading scientifically [although some have seen a real significance in the resemblance; no connoisseur of ingenious casuistry should miss reading Crookshank (1931)]; but also because the term can be offensive to true Mongols (amongst who are numbered several leading human cytogeneticists).

However readers will nevertheless notice that the term mongol is still used in the following pages. Anyone who writes on the subject becomes rapidly aware that there is no other convenient short term available for a sufferer from Down's syndrome. Perhaps we should speak of a "Down". The two most obvious characteristics of Down's syndrome are firstly an unusual appearance which is so consistent that all sufferers could almost be taken for siblings, and secondly and invariably, mental deficiency.

2. Incidence of Down's Syndrome

The incidence of Down's syndrome at birth is about 1.5/1000; this figure is based on a number of separate series of records, mainly from maternity hospitals (the condition can and should be diagnosed at birth) and on one complete survey in Victoria, Australia, by

Collmann and Stoller (1962) which showed that between 1942 and 1957 1134 cases were found in 780,168 live births (1.45/1,000); this relates to populations of European stock only; although the condition occurs in all races, comparable figures are not available for any other ethnic group. There is little point in suggesting confidence limits for the figure given, since there are reasons to suppose that the expectation of a mongol child is not absolutely fixed for all women at all times; it will be affected by the age structure of the population and possibly by epidemiological considerations [Collmann and Stoller (1962) found geographical concentrations of births in particular years, unrelated to age distribution]. The question of incidence will be considered more fully later when aetiology is discussed. Incidence in the total population is considerably lower than that at birth since, as will be seen later, there is a high mortality throughout childhood, particularly early; various estimates have been made, most of which are now out of date since the population incidence is almost certainly rising all the time with improved medical care; but the incidence is probably at least 1/3000.

3. Features of Down's Syndrome (Figs 51 and 52)

(a) Physical features. These have been extensively described (for reviews see Penrose and Smith, 1966; Benda, 1946, 1960) and may be summarized by saying that on careful examination almost every tissue in the body can be shown to be slightly defective, particularly in respect of growth and development. This is perhaps most obvious in the head region. The brain is always small (weighing on average about 80% of the normal weight) and the cranium which contains it is therefore also small, and rounded, with a characteristically flat back. Growth of all the skull bones is defective; the undergrowth of the cartilage in the base of the skull leads to the small, under-developed-looking nose, with a flattened bridge and "epicanthic folds" of skin medial to the eyes. The teeth are late erupting, irregular and sometimes missing. The tongue does not share the defective growth of its surroundings, and often protrudes; it is usually coarsely fissured on the dorsum. The external ear (auricle) is usually small, with, typically, an angular, overhanging helix; but it may be normal in appearance and larger than usual. In the eyes, the iris is usually speckled (Brushfield's spots) due to imperfect development; lens opacities are common. The hair is, interestingly, always straight.

In the post-cranial skeleton generalized undergrowth of cartilage leads to stunting of growth (Roche, 1965); adult mongols are

Fig. 51. A child with Down's syndrome. Characteristic features which can be seen include round head, straight hair, snub nose, epicanthic fold and strabismus.

invariably short. The stunting is particularly obvious in the extremities; the digits are short, and the fifth finger is often short and incurving, with a short or even absent middle phalanx. The generalized impression of deficient growth at the extremities of the limbs, nose, etc., caused the name "acromicria" to be coined by Neumann (1899). The skin generally is dry and coarse; there are abnormal dermatoglyphics (see Chapter 12).

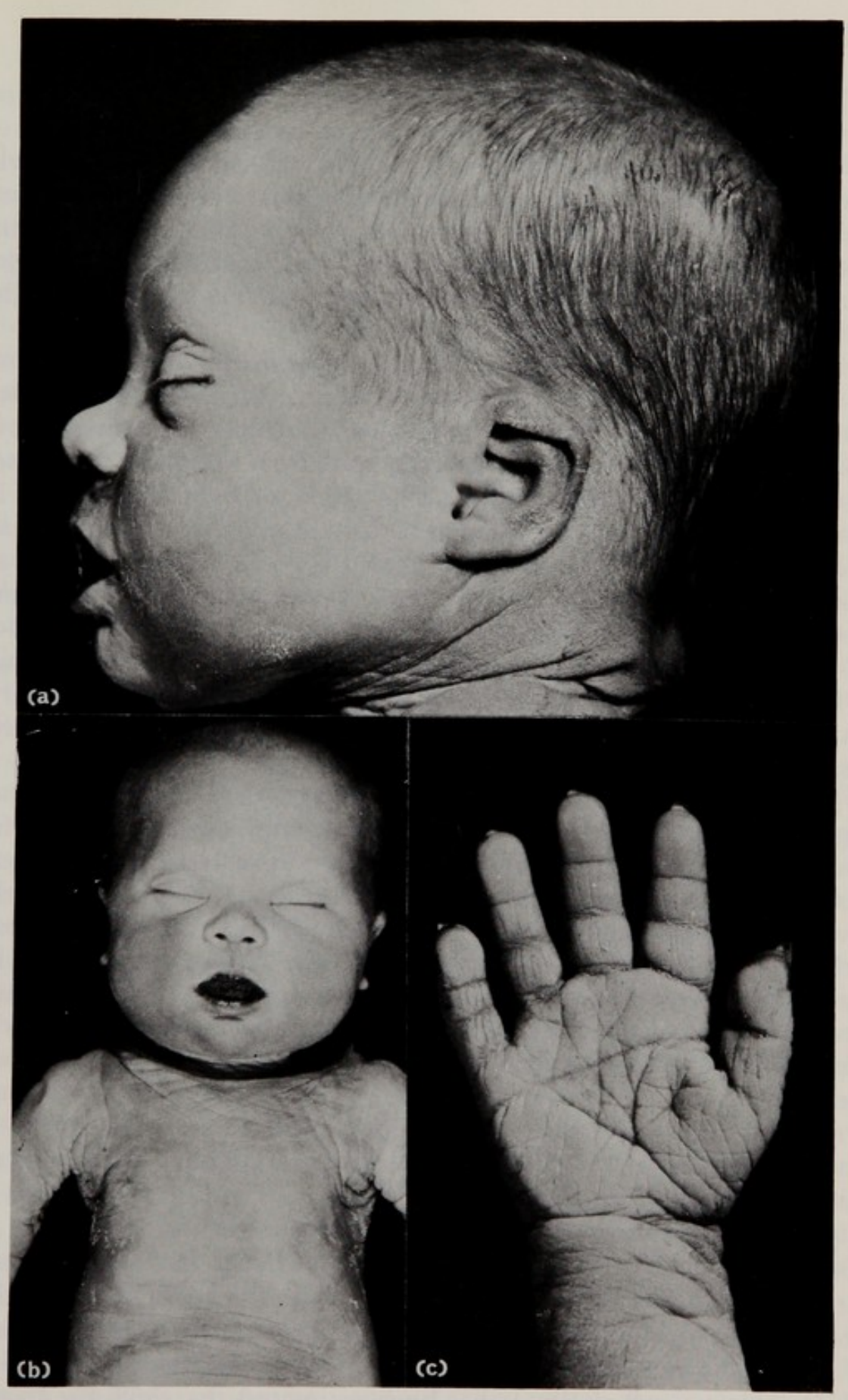

Fig. 52. A stillborn infant showing features of Down's syndrome. (a) Profile, showing flat back of head, small nose, malformed pinna of ear. (b) Full-face view, showing mongolian slant to palpebral fissure, epicanthic fold and enlarged tongue. (c) Hand, showing "simian" (transverse palmar) crease and short little finger.

Mesodermally-derived tissues are not spared. Cardiac anomalies are very common (occurring in some degree in probably at least 50% of sufferers, and accounting for much of the mortality in early childhood). There is a low absolute and relative lymphocyte count, and this may relate to an increased susceptibility to infections, another contributory cause of the high childhood mortality. Endocrine glands generally have been described as being hypoplastic. The muscles are hypotonic.

Sexual development, like all other developmental features, seems to be somewhat defective, but not totally so. In males undescended testes are common; libido is diminished, but spermatogenesis does take place and semen with a reduced sperm count can be produced; but there is no proven case of a male mongol fathering a child. In females secondary sexual characteristics are usually late in appearance and under-developed. Menstruation is often absent. Despite all this, a small number of mongol females have become mothers, and about half of their offspring were themselves affected, giving proof of the inevitable non-disjunction which might be expected to occur.

In short, every system is defective, but usually not profoundly. The only system in which this mild underdevelopment is always disastrous in effect is the nervous system.

(b) Mental features. All grades of mental deficiency are found, but the I.Q. is usually between 20 and 50, and hardly ever rises far above the latter figure. This means that the most intelligent mongols can just learn to read and do manual tasks (such as woodwork) involving a little skill. Most are incapable of this, and many are too deficient (for example) even to learn to talk. Personality tends to be stereotyped, but often in a satisfactory way; thus mongols are often described as being lively and imitative, placid, well-mannered, gregarious, playful and affectionate, fond of music, and so on. There are exceptions to every rule, and every sufferer from Down's syndrome is genetically unique, but nevertheless the physical resemblance between cases seems to be reflected in a general resemblance of personality.

(c) Expectation of life. Penrose estimated the life expectancy in 1947 to be 12 years (improved from 9 years in 1929). The latest estimate, on Australian children (Collmann and Stoller, 1963) is 18. The increase is due to the remarkable improvement in the standard of care of both mentally defective and normal children, introduction of antibiotics, and so on. It has been estimated that 30% die in the first month, 50% are dead by one year, and 60% by 10 years.

(d) General teratological aspects. In terms of human growth and development generally, Down's syndrome must be considered a very

mild abnormality. A genetically determined condition which is compatible with survival to adult life, with adequate functioning of body systems generally, and with an I.Q. of up to 60 is, however much one may feel instinctively that the abnormalities are gross, a very mild genetic deviation from the normal. The mongol must be regarded as a "near miss" at a normal individual. This in itself indicates that the chromosome concerned (which is the smallest of the autosomes) is relatively inert genetically. It is interesting that fluorescent techniques show that the chromosome involved (conventionally called 21) is more fluorescent than the other G group chromosome; if fluorescence is an indication of relative genetic inactivity this might have been predicted.

It is not known how the presence of an additional chromosome in all cells of the body produces the generalized abnormalities found. It has been suggested that the effects are due to the presence of the additional genetic loci on the chromosome concerned; some support for this has been suggested by raised levels of some enzymes, such as alkaline phosphatase, in the leucocytes of sufferers. Raised enzyme levels are not however found in cultured fibroblasts (Nadler *et al.*, 1967) and there is no definite evidence that gene action is responsible for the abnormalities found; indeed no genes have yet been located with certainty on chromosome 21. It is possible that all the changes found could be explained on the basis of a slight disturbance of the kinetics of cell division due to the extra chromosome, particularly affecting cells at a stage of rapid division in the early embryo. The development of the nervous system in particular, with the accurate interconnections between neurones which have to be made, must be dependent on the most exact timing of all the events involved; it is not surprising that this is the most sensitive of all body systems to minor disturbances of development.

4. Genesis of Down's Syndrome

When considering why cases of Down's syndrome arise it is necessary to consider first what the underlying physical abnormality is; secondly what the mechanisms are which may have produced this abnormality; and thirdly, what it is that has set these mechanisms in motion. This last is the true *aetiology* of the condition, and it must be admitted that as yet little is known of the factors involved.

Much however is known about the genetic abnormality which causes Down's syndrome, and of the actual mechanisms of its production. It is generally agreed that the underlying genetic abnormality is a triplication of the material in the smallest autosome (No. 21) (Fig. 53). All cases of Down's syndrome so far reported

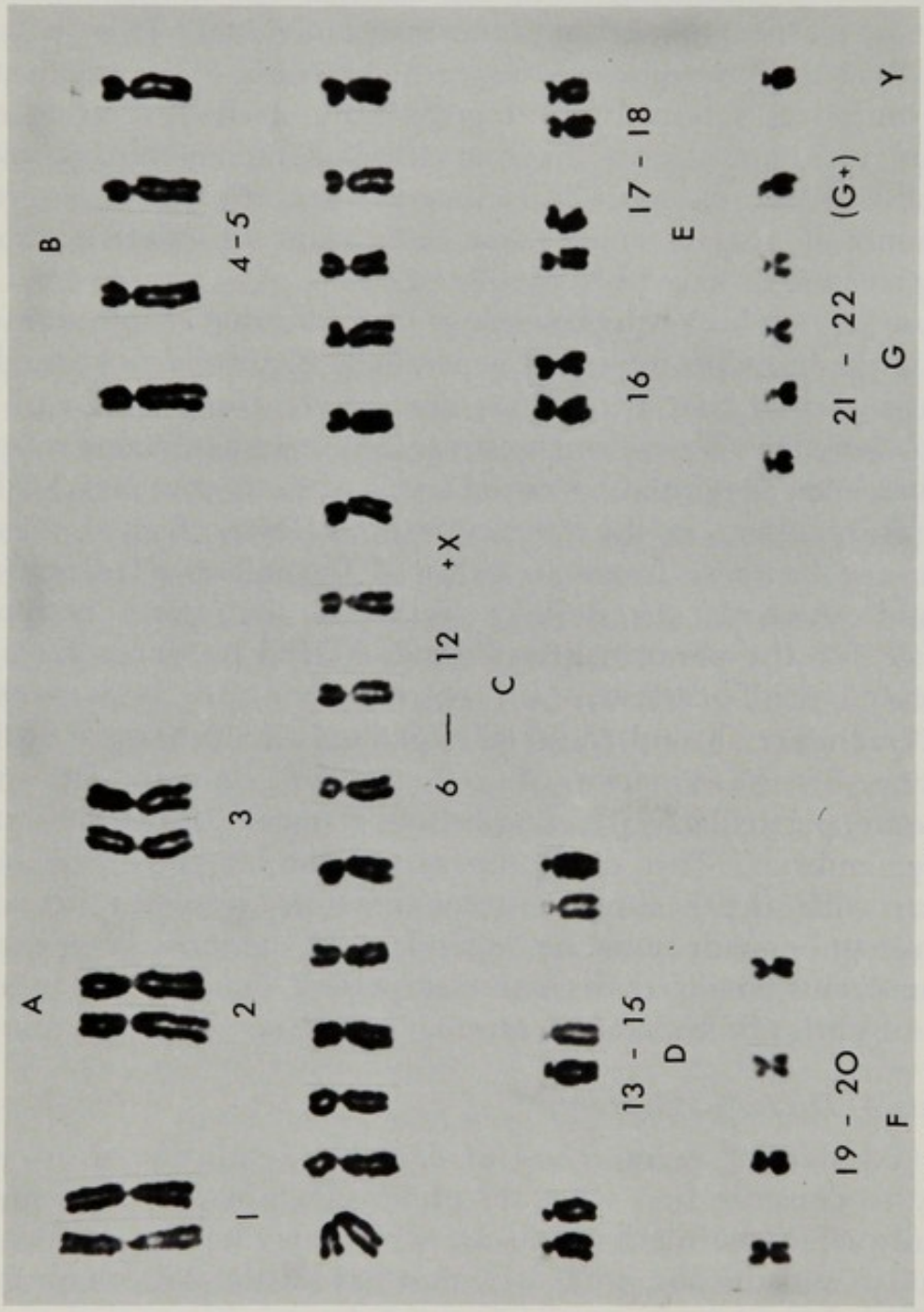

Fig. 53. Karyotype of a child with Down's syndrome, showing trisomy for a G group chromosome.

have in all, or some of their body cells, this extra genetic material, either in the form of a complete extra chromosome (trisomy 21) or of the major part of this chromosome, either free or translocated to another autosome (almost always of the D or G groups). Simple trisomy 21 is by far the commonest cause, and was first demonstrated by Lejeune *et al.* (1959)—the first major condition in man which was shown to be due to an abnormality of the chromosomes.

Before Lejeune *et al.* (1959) made the discovery, the cause of Down's syndrome was unknown. Many ideas were put forward to explain the genesis of the condition; for example syphilis, tuberculosis, parental alcoholism, endocrine deficiencies (in particular hypothyroidism), use of chemical contraceptives, faulty implantation of the embryo, degeneration of the ovum, and the already-mentioned atavistic "throwback" to an ancestral Mongoloid ancestor, were all suggested. The only causally-related factor which was clearly demonstrated at this time was a relationship to maternal age; it was very clear from the researches of Jenkins (1933) and others that the liability to have a child with Down's syndrome increased with maternal age.

However it was also becoming apparent to certain far-sighted workers that a genetic factor was involved, certainly in some cases (e.g. Penrose, 1954). Mere incidence of the condition in more than one member of a family is not enough to suggest a genetic causation when a condition is as common as Down's syndrome; but three facts indicated that inheritance of the condition might sometimes occur. The first was that twin studies showed clearly that dizygotic twins were found to be unequally, and monozygotic twins equally affected; the second that the syndrome could on occasion be transmitted directly from mother to child; and the third that when more than one member of a family was affected the dependence upon maternal age was weakened (Penrose and Smith, 1966). Study of cytogenetic abnormalities found in Down's syndrome has explained all these phenomena.

(a) Cytogenetics of Down's syndrome. There are four major cytogenetic types of Down's syndrome.

(i) Standard trisomy 21 (47,XX or XY,G+) (Fig. 53). This accounts for about 95% of cases. Every cell in the body which can be examined has an extra small acrocentric chromosome which has the following characteristics: it appears identical morphologically with the smallest autosome (conventionally called No. 21), which is later-labelling, more often satellited, and more intensely fluorescent staining in its long arm, than the other G group chromosomes.

(ii) 46,XX or XY,D−,t(DqGq)+. This karyotype occurs in probably rather less than 2% of cases (1.7% of 1103 cases studied by Richards, 1967). Phenotypically it is effectively indistinguishable from standard trisomy 21 (in theory there might be small phenotypic differences since in the formation of this type of "centric fusion" or Robertsonian translocation a small centric fragment is normally eliminated which will contain genetic material from one or both of the chromosomes involved: Gibson and Pozsonyi (1965) have found evidence that translocation mongols of this type are slightly less severely affected than standard trisomic mongols: the fact that phenotypic differences are not pronounced reinforces the view that material in the region of the centromeres of the acrocentric chromosomes, and in the short arms, may be genetically inert or redundant.) The importance of this variety of karyotype is that the abnormality may be inherited from a parent who may be phenotypically normal but have an abnormal but balanced karyotype which includes the translocation chromosome. This accounts for a number of the reported instances of familial mongolism. However, most mongols with this karyotype appear sporadically and have parents with normal karyotype (Polani *et al.*, 1965). Transmission of the abnormality is discussed in greater detail later.

(iii) 46,XX or XY,G−,t(GqGq)+. This karyotype occurs with a comparable frequency to the previous one, but less commonly (in perhaps slightly over 1% of cases). Again, affected individuals may not be quite so severely affected phenotypically as standard trisomic mongols (Soudek *et al.*, 1968), although evidence on this is scanty. This translocation accounts for some examples of familial mongolism. There are two varieties of this translocation, according to whether it is a 21/21 or a 21/22 centric fusion. The importance of distinguishing the two lies in the fact that a parent with a balanced 21/21 translocation is incapable of having normal offspring (see later). The new fluorescent and banding techniques should allow the two varieties to be distinguished without difficulty.

(iv) Mosaic karyotypes (mixoploidy). These are normally 46,XX or XY/47,XX or XY,G+. Mosaic mongols are rare, accounting for perhaps 2.5% of all mongols (Richards, 1967); this figure is likely to be an under- rather than an over-estimate, since some cases may go undetected, especially if one of the cell lines is infrequent or absent in some tissues.

The phenotype of such mosaics varies, as might be expected, within a spectrum from virtually normal to typical mongol, in relation to the proportion of the trisomic cell line in the body as a

whole, and in particular tissues. Penrose (quoted in Penrose and Smith, 1966) gave means for the proportion of normal and trisomic cells in 17 mosaic mongols, finding on average that some 25% of leucocytes but over 50% of fibroblasts were trisomic. It seems to be a general rule that fewer trisomic cells are found in the blood than in fixed mesodermal tissues.

Taylor (1968b) has shown (by longitudinal studies of the proportions of cells of the different cell lines present at different periods), that vigorous cell selection takes place in the stem cells of peripheral blood leucocytes in infants with mosaicism of this type and that in any given child either the normal or the trisomic cell lines tend to increase in proportion to the whole; once a direction is established it seems to be maintained. This is not however found in adult mosaics, in whom, presumably, an equilibrium has been established.

Apart from mosaics who show frank mongolism, there are also individuals in the population who have trisomic cells in some part of their body, but are phenotypically normal (or almost so). If trisomic cells were to be confined to such tissues as the ovary, or the nervous or muscular systems, they could not be detected by normal cytogenetic methods since there is no ready way of obtaining dividing cells from these tissues. Nevertheless Penrose and Smith (1966) have detected dermatoglyphic anomalies (*q.v.*) in some 10% of mothers of mongols, which they consider may be evidence of a latent but otherwise undetectable mosaicism, which may have been present in the ovary and given rise, by inevitable non-disjunction, to trisomic gametes and hence offspring with Down's syndrome. Mikkelsen (1967), studying a series of families in which young mothers had had offspring with Down's syndrome, found that in 62 regular trisomy 21 mongols, the age of the maternal grandparent was significantly higher than that of the paternal grandparent, suggesting that a fair proportion of these young mothers were mosaics, following non-disjunction in a trisomic zygote (see p. 231).

Mosaicism may also be found in the fathers of mongols. Hsu *et al.* (1971) have recently described three fathers of mongols, all of whom were under 30 years old, and were mosaics for trisomy 21; the mosaicism was demonstrated in both fibroblasts (from skin and testis) and lymphocytes in two of the three; in the third, mosaicism was demonstrable in fibroblasts but not in lymphocytes.

(v) Other karyotypic variants associated with Down's syndrome. These are all exceedingly rare in comparison to the four main varieties which have been described, but have been occasionally

described in the literature. Other reciprocal translocations leading to offspring with unbalanced karyotype can occur (e.g. 21q−:22q+ translocations described by Cohen and Davidson (1967), and Lejeune *et al.* (1965), a B/G translocation (Aarskog, 1966) and a 2/G translocation (Miller *et al.*, 1970). A number of cases have been described where there are 47 chromosomes, the additional one being smaller than a G group chromosome but presumed to have originated from one by deletion (partial mongolism; e.g. the cases of Dent *et al.* (1963), Kumbnani and Pfeiffer (1969) and Subrt and Prchlikova (1970)); such patients show most but not necessarily all the stigmata of mongolism. Mosaicism involving a line with a ring chromosome (45,XX,G−/46,XX Gr; Blank and Lorber, 1969) and involving two abnormal chromosomes 21, one an isochromosome (46,XY,21qi/46,XY,21p−; Atkins and Feinold, 1969) has been described. But these are all isolated cases and of great rarity.

(vi) Down's syndrome in association with other chromosomal anomalies; double trisomies. The first case of double trisomy (48,XXY,G+) was described by C. E. Ford *et al.* (1959) and since then several have been described. Double trisomy is relatively rare in Down's syndrome (Taylor, 1969). The commonest is 48,XXY,G+, estimated to occur once in 11,000 live male births; 48,XXX,G+ is very rare as is 48,XYY,G+ (described by Uchida *et al.*, 1966).

(b) Mechanism of genesis of the various cytogenetic varieties of Down's syndrome. (i) Standard trisomy 21. The immediate cause of trisomy 21 affecting all the cells in the body must be a non-disjunctional event. While this could occur in the fertilized zygote at the first cleavage division with loss of the 45,G− line, or possibly even later in early development, this must be an excessively rare event, and for all practical purposes non-disjunction in one or other parent may be assumed.

The only factor which has long been known (Jenkins, 1933) to be associated with an increase in the likelihood of the birth of a mongol is maternal age. This has been analysed in great detail by Penrose (reviewed in Penrose and Smith, 1966) and it has been shown without doubt that the danger of giving birth to a mongol child increases with the age of the mother, slowly at first, but with increasing rapidity after the age of 30. The risk is summarized in Fig. 54, whence it can be seen that a woman under the age of 20 has only a 1/2000 chance of having a mongol child; the risk rises to about 1/1000 at age 30, and thereafter steeply, until at 40 it may be as high as 1/100, and at 45 or over perhaps as high as 1/50. Penrose states that the incidence rate after the age of 30 almost behaves as a

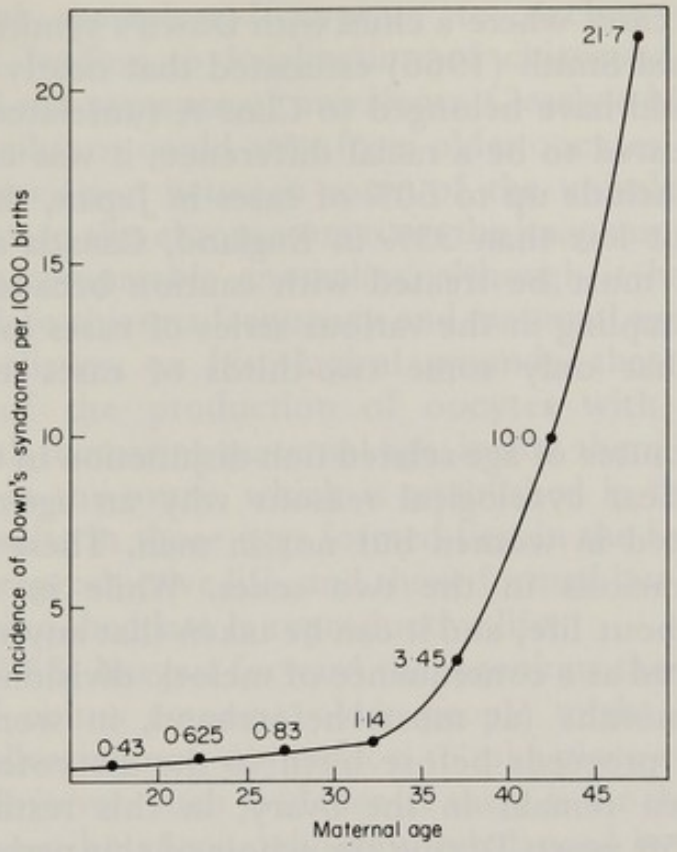

Fig. 54. Birth incidence of Down's syndrome at different maternal ages (from the figures of Collmann and Stoller, 1962).

logarithmic function (increasing exponentially like compound interest) although the rate tends to flatten off somewhat at the age of 40.

It has also been shown quite plainly that the age of the father is not a relevant factor. Of course, older women will, on average, have older husbands, but if this factor is corrected for, it can be shown that there is no significant paternal age-effect.

It is of course clear that not all cases of Down's syndrome are related to maternal age. Cases due to inevitable non-disjunction (due to a mother who is trisomic either *in toto* or in the ovary), or arising as a result of an unbalanced translocation (see below) or as a result of non-disjunction in the father, or from other causes less well known, will not be related to maternal age. Thus two classes of cases may be defined; those causally unrelated to maternal age, called by Penrose Class A, and those related to maternal age (Penrose's Class B). Calculation of the exact proportion of the two classes amongst all sufferers from Down's syndrome is not quite straightforward, but one method which may be used to make an estimate (and probably the simplest) is to assume that in the youngest mothers (those in their teens) there is no age-related effect. Using this method on a

sample of 9441 cases where a child with Down's syndrome had been born, Penrose and Smith (1966) estimated that nearly two-fifths of all the cases could have belonged to Class A (unrelated to maternal age). There appeared to be a racial difference; it was estimated that Class A might include up to 50% of cases in Japan, the U.S.A. and the U.S.S.R., but less than 33% in England, Canada and Formosa. These estimates must be treated with caution because of possible differences in sampling in the various series of cases included, but it remains clear that only some two-thirds of cases are related to maternal age.

(1) Possible causes of age-related non-disjunction in women (Class B). There are clear cytological reasons why an age-related effect might be expected in women but not in men. These relate to the differences in meiosis in the two sexes. While in men, meiosis proceeds throughout life, and it can be taken that any spermatozoon has been produced as a consequence of meiotic divisions taking place within a few months (at most) beforehand, in women the first meiotic division proceeds before birth to the dictyotene stage, and the oocytes then remain in the ovary, in this resting stage, for anything up to 50 years. During the whole of this period they are at risk, either from a simple ageing process, or from external environmental influences; background and therapeutic irradiation, drugs, infections and so on.

Any or all of these factors might be involved in producing non-disjunction. Simple ageing of the oocyte may be important; Penrose and Smith (1966) suggest that the exponential rise in incidence with increasing age could well be explained by a series of independent accidents occurring and causing, for example, degeneration of the spindle. Another mechanism which has been suggested is an age-related failure of breakdown of the nucleolus during cell-division, which might lead to excessively prolonged association of acrocentric chromosomes and so to non-disjunction.

Many environmental influences have been suggested as possible causes of non-disjunction. These will be considered under Class B, but it must always be borne in mind that if environmental causes can cause non-disjunction, there is likely to be some age-related effect because the longer the life, the greater the risk of exposure at some time. The same consideration could apply to an inherited tendency to non-disjunction if it is a significant causal factor.

Edwards has recently put forward another possible cause of age-related non-disjunction (reviewed by Edwards and Fowler, 1970). In mouse oocytes, the number of chiasmata seen per cell at

diakinesis and metaphase-I appears to decline with increasing maternal age, leading to localization of chiasmata to the ends of bivalents, and the presence of univalents. Clearly a high frequency of non-diploid embryos could arise from older oocytes, especially if the secondary association between some of the univalents was insufficient to orientate the chromosomes on the anaphase spindle. Human oocytes show comparable anomalies, although it has not yet been possible to relate chiasma frequency and maternal age.

Edwards believes, on histological grounds, that the most likely explanation of the production of oocytes with a low chiasma frequency with increasing maternal age, is that there is a "production line" of eggs in the ovary, which is established in the foetal period, and which results in those eggs formed first in the foetal ovary being shed early in reproductive life, and those formed last (and with fewer chiasmata) being shed late in reproductive life.

German (1968) has put forward the ingenious theory that reduced frequency of coitus amongst older people might lead to delayed fertilization allowing non-disjunction to take place at second meiotic metaphase. However, such evidence as there is for this theory, which is statistical, can be adequately counterbalanced by other statistics; for instance Cannings and Cannings (1968) suggest that the age-dependency for frequency of coitus is not sufficiently closely related to that for the incidence of Down's syndrome to support the hypothesis.

However it must be admitted that all theories yet put forward to explain "degeneration of the ovum (or, more correctly, oocyte)" are based on circumstantial evidence and lack direct proof.

(2) Possible causes of non-age-related non-disjunction in women (Class A). These comprise unbalanced translocations involving chromosomes 21, inevitable (secondary) non-disjunction, non-disjunction in the father, genetically determined non-disjunction, and environmental influences causing non-disjunction.

Unbalanced translocations will be considered in the next section. Inevitable non-disjunction has already been mentioned. It may occur in trisomic mothers, but this is very rare, for obvious reasons, and need not be further discussed here. Much more important is the fact that it may occur in women who are mosaic for trisomy 21; and if the mosaicism affects only the ovary, or that and some but not all tissues, these women may have a normal, or almost normal, phenotype. The condition may only be suspected because of the occurrence of more than one affected child. Suspicions may be reinforced by the finding of abnormal dermatoglyphics (*q.v.*) in these

mothers, and Penrose (1965) has estimated on the basis of dermatoglyphic microsymptoms that 10% of all mothers of mongols may be normal/trisomic mosaics. Several such cases have been cytogenetically proven (Penrose and Smith, 1966).

Of course, trisomy can also occur in the father; but as mentioned earlier, no case of a fully trisomic male mongol becoming a father has yet been reported. Normal/trisomic mosaicism has only recently been demonstrated in fathers of trisomic mongol children (Hsu *et al.*, 1971), which suggests that such paternity must be a rare event, and that there may be some prezygotic selection against trisomic sperm. However, Penrose (1965) estimates, again on the basis of dermatoglyphic microsymptoms, that 1% of mongol births may be a consequence of paternal normal/trisomic mosaicism.

Genes causing non-disjunction in human families have not been proved to exist, but there is some circumstantial evidence to suggest that they occur. Such genes are known in *Drosophila* (Lewis and Gencarella, 1952). In maize the gene "sticky" (Beadle, 1933) can be responsible not only for meiotic non-disjunction, but also for aberrations in somatic mitoses, giving the possibility of mosaicism as an inherited trait.

A number of human families have been described in which the occurrence of several different non-disjunctional anomalies may suggest a genetic cause such as the family described by Miller *et al.* (1961) with a 49,XXXXY male, two 47,XX,G+ mongol girls and a boy with leukaemia. However, isolated instances of families like this might be expected to occur sporadically through the operation of chance. But the likelihood that they are manifestations of genes favouring non-disjunction is greater if the mothers concerned are young. Stronger evidence is provided by the remarkable families described by Drets *et al.* (1970) which showed normal/partial trisomy 16 mosaicism in four members, and by Green *et al.* (1968), with 13-15 chromosomal mosaicism in two generations.

Further, but perhaps less strong, evidence is provided by the relative incidence of double trisomies in patients with Down's syndrome. Taylor and Moores (1967) give the incidence of Down/Klinefelter syndrome as 1/11,000; previous estimates of the incidence of chromatin-positive mongols suggested that 1-1.5% of mongols might be so afflicted (giving a birth incidence of 1/45,000-60,000 approximately). The expected incidence if the two non-disjunctional events are totally unrelated would only be 1/360,000 (Penrose and Smith, 1966). Of course if environmental influences cause trisomy, then double trisomies might be expected to

occur more frequently than this, so the incidence cannot be taken as proof of a genetic cause.

However, if non-disjunction were caused by maternal homozygosity for a rare gene it would be expected that an increase in consanguinity among maternal grandparents would be found. This does not appear to be the case in large series investigated by Penrose (quoted in Penrose and Smith, 1966) and Forssman (1965), so if such a gene is concerned it must be common. Juberg and Davis (1970) also found, in a study of all mongols born in an inbred religious isolate (the Old Amish) with detailed and accurate genealogical records, that there was no evidence of greater inbreeding amongst families with mongol children.

Despite all this conflicting evidence it is difficult to avoid a feeling that a genetic factor may be involved in some instances of non-disjunction in man.

A number of environmental agents have been suggested as possible causes of non-disjunction (reviewed by Penrose and Smith, 1966). Support is lent to the general conception by indications that epidemiological clustering of mongol births occurs, both in time and geographically (Stoller and Collmann, 1965). The only two environmental factors for which there is any valid evidence are maternal irradiation and maternal infection. Numerous other possible causes such as maternal illness during pregnancy, hormonal disturbances, emotional stress, fluoride in the water, and so on, have been postulated, without sufficient evidence. The evidence that irradiation before or during pregnancy is a contributory cause is equivocal. Carter *et al.* (1961) considered that its significance had not been established. Other workers have from time to time found some statistical support, not strong (e.g. Sigler *et al.*, 1965); recently Stevenson *et al.* (1970) concluded that there is no evidence that maternal diagnostic irradiation before conception has any influence on the incidence of subsequent mongol births.

There is rather more evidence to suggest that infections may play a part in causing non-disjunction. The main evidence concerns the virus causing infectious hepatitis. It is known that an agent in the plasma of patients with hepatitis can cause chromosome breaks in human leucocyte cultures (El-Alfi *et al.*, 1964). Stoller and Collmann (1965) in the course of their extensive study in Australia observed that the fluctuations in the incidence of hepatitis were closely related with incidence of mongol births subsequently, over a period of 12 years. If this virus is capable of causing such an effect, there is no reason to suppose that others may not also.

To summarize this section on Class A (non-age-related) non-disjunction the opinion of Penrose (in Penrose and Smith, 1966) is that of the 40% of mongol births which may fall into this class, roughly one quarter (10%) is due to each of the four main categories quoted: inevitable non-disjunction (usually associated with mosaicism in the mother), unbalanced translocations involving acrocentric chromosomes, a familial predisposition to non-disjunction (or mosaicism), and environmental causes. To these must be added a proportion of cases due to chance paternal non-disjunction. Although there is little doubt about the total proportion of age-unrelated cases, this breakdown into different causal factors must be regarded as very tentative.

(ii) Down's syndrome due to unbalanced karyotype following centric fusion ("Robertsonian translocation") involving chromosomes of the D and G group: 46,XX or XY,D−,t(DqGq)+. The first cases of Down's syndrome associated with these types of translocation were published in 1960; Polani *et al.* (1960) described a case of D/G translocation mongolism, which was shown later (Carter *et al.*, 1960) to have been inherited from a parent with a balanced translocation. Penrose *et al.* (1960) were the first to demonstrate familial transmission, in a similar case. Since that time many similar examples have been described (Penrose and Smith, 1966).

While it has been shown by both autoradiographic and fluorescent staining techniques that the G group chromosome involved is the same as that involved in standard trisomic mongolism, not enough cases have yet been described to give reliable figures for the relative frequencies with which the three D group chromosomes take part in these translocations. However, it is fairly clear that chromosome No. 14 is the commonest, and that chromosomes 13 and 15 are less often involved. Miller (1970) found that in 17 cases studied autoradiologically by various workers, chromosome No. 14 was involved in 13, No. 15 in 3 and in one the chromosome was either 13 or 14. O'Riordan *et al.* (1971) found, by fluorescent staining in five cases, that chromosome No. 14 was involved in four, and No. 13 in one.

D/G translocation mongols may appear *de novo* or as a consequence of one or other parent having a balanced karyotype containing the translocation, and producing unbalanced gametes. The majority of cases are in fact found to have parents with normal karyotypes (Polani *et al.*, 1965); these parents are presumably undetected mosaics with some cell lines, certainly in the gonad, and possibly elsewhere, which contain the translocation.

The usual karyotype in these cases is 46,XX or XY,D−,t(DqGq)+. The appearance is that a D and a G chromosome have fused to form a new metacentric chromosome, hence the common name "centric fusion", which is given. This type of translocation was first described by Robertson (1916), in particular relation to the evolution of karyotypes amongst different species of animal by means of a reduction in the total number of acrocentric chromosomes present, associated with an increase in the number of metacentrics present (see White, 1954), presumably through fusion of pairs of acrocentrics to form single metacentrics. The process is well seen in its evolutionary form in the primates; lower primates tend to have a fairly large number of acrocentric chromosomes, while higher primates have a reduced total chromosome number associated with a decrease in acrocentrics and an increase in metacentrics. The term "Robertsonian translocation" is favoured by many to describe this process both in evolution and in the corresponding type of translocation in man. Of course, when the translocation occurs, it is presumably a reciprocal one which gives rise to a new translocation chromosome plus a centric fragment containing little but the centromere of one of the two chromosomes concerned (Fig. 49). For some unknown reason this centromeric fragment is usually lost, although such fragments have been seen in phenotypically normal persons and have been ascribed to a Robertsonian translocation occurring in an ancestor (e.g. Hoehn *et al.*, 1970). Possibly, however, the process is not a reciprocal translocation, but a true fusion involving a break at the centromere of both chromosomes concerned, followed by fusion, and loss of the other two half-centromeres. But there is really no evidence on this point.

The reason for the frequency of this type of translocation in man is not hard to see; it relates to the proximity of the chromosomes concerned because of their common association with the nucleolus at their centromeric ends, which means that if breaks occur in two of these chromosomes at the same time (which could be due to one mutagenic event, such as the effect of ionizing radiation), misjoining is easy.

The main practical interest in these translocations, for the cytogeneticist, lies in deciding what advice should be given to potential parents who possess a balanced translocation of this kind. Despite a considerable corpus of knowledge on the behaviour of this and other types of translocation chromosome at meiosis, which will, because of its interest, be considered separately later, it is generally agreed that such advice can only be given on an empirical basis,

relying on accumulated data from two sources: experience of what happened in all known instances of the particular variety of translocation; and experience (if any) of the behaviour of the translocation in the actual family involved. The latter will, if available, be the best guide, since it must always be remembered that every translocation is different, since it involves unidentical genetic material, and may behave differently from another apparently identical translocation in another family.

Just occasionally a family is found in which a fairly large number of members carry a translocation. A good example is the family described by Shaw (1962), in which twelve sibships were found in which one or other parent carried a D/G balanced translocation arising from a common origin. These sibships contained 48 phenotypically normal offspring and six mongols with a unbalanced karyotype including the translocation. No mongols were found among the 18 offspring of five male balanced translocation carriers. On this basis it would probably be justifiable to give the following counsel to a member of the family carrying the balanced translocation, and wishing to have children; that if they were female the best estimate of the risk of a mongol child would be 12%; and that if they were male, the risk would be rather less than this but unquantifiable. Unfortunately (or perhaps fortunately) such families are very rare indeed.

In the absence of familial data, advice must be based on knowledge of the behaviour of such translocations in the population at large. Hamerton has studied this subject for some years, and produced a series of reviews (Hamerton, 1966, 1968, 1970). The subject is bedevilled by the great bias introduced by the fact that most families are ascertained through an abnormal proband, and thus families with translocations, but with no phenotypically abnormal members, are not adequately represented; such families are occasionally turned up during routine screening of various populations, but not often enough, since the condition is so rare. Ideally only such families should be studied, giving a true *prospective* study; but inevitably studies are mainly *retrospective,* based on families with an affected proband. Only in 13 of 41 families studied by Hamerton (1970) was the proband ascertained at random.

Hamerton estimates on this data that the risk of a heterozygous mother with balanced translocation, t(DqDq) or t(DqGq) having an affected child is 10-15%; the risk for a heterozygous father is about 5%. This does not of course take into account which D chromosome is involved in the translocation. There could be differences in behaviour according to whether No. 13, 14, or 15 is involved.

Balanced heterozygotes can, in practice, produce three types of gamete (other types are possible in theory-see p. 000), which will give three types of offspring: normals who are karyotypically normal, phenotypic normals who have a balanced translocation like their parent, and unbalanced offspring who are in effect trisomic for the G chromosome (another theoretical possibility, trisomy for the D chromosome, has not yet been seen), and *a priori* these three types should be present in equal numbers. In fact Hamerton (1968) found that only 6.1% of progeny had Down's syndrome (against the expected 33%); 10% of progeny of female heterozygotes and 2.4% of progeny of male heterozygotes. Of the remainder, rather more were balanced heterozygotes than normals (54% to 40%). Because of the heavy correction necessary to eliminate bias, these figures must be regarded as tentative and subject to modification in the light of further data.

No increase in the level of spontaneous abortions as compared with that of the general population was found in this series; such an increase might *a priori* be expected as a result of the various types of inviable gametes which could be produced (see p. 168).

The possible introduction of bias, because families are ascertained through an unbalanced proband, is well illustrated by recent findings by Jacobs *et al.* (1970). In families ascertained through a proband with a balanced translocation *no* unbalanced progeny were found from 29 such probands; but where ascertainment was through an unbalanced proband 15% of the liveborn children of female carriers, and 8% of the liveborn children of male carriers, were found to carry an unbalanced translocation. 10% of the progeny of female carriers of Robertsonian translocations of the t(DqGq) type, and 2% of the progeny of male carriers, had an unbalanced translocation.

(iii) Down's syndrome due to unbalanced karyotype following centric fusion of the G/G type; 46,XX or XY,G−,t(GqGq)+. Fraccaro *et al.* (1960) described the first case with this karyotype, inherited from a father with a balanced translocation. Since then a number of examples have been described. Both 21/21 and 21/22 translocations have been described, and these will behave differently. 21/21 balanced translocation carriers are incapable of producing normal offspring; the gametes necessarily produced must give rise to either trisomic or monosomic zygotes. Such families have been described (e.g. De Capoa *et al.*, 1967). 21/22 balanced heterozygotes can on the other hand produce normal, carrier or mongol offspring, as for D/G translocations (as well as other possibilities such as trisomy 22, which have not in fact been found).

G/G translocations were found by Richards (1967) to be

responsible for 1.4% of 1103 cases of Down's syndrome. The author has not been able to find data on the relative frequencies of the two types of G/G translocation but these will no doubt soon be forthcoming as a result of the introduction of fluorescent staining techniques.

21/22 translocations seem to differ in behaviour from D/G translocations in that they are more commonly transmitted through the father (Penrose and Smith, 1966). Penrose found a significant increase in paternal age (mean age of fathers at birth of probands was 42.5 years, 10.8 years above the controls. Even in cases where both parents have normal karyotypes the father's mean age (35.6 years) was still elevated.

In a family reported by Shaw (1962), of 17 offspring of segregating marriages, two were mongols, eight carriers, and seven karyotypically normal. In Hamerton's (1968) series, of a total of 30 offspring from such marriages in 13 families, two were mongols, 13 carriers and 15 normal (figures corrected by removal of probands). Here the mongols were born to female heterozygotes, and Hamerton estimates the risk of an affected child at under 10% (on this small series), with no definite evidence for a different risk to male and female carriers.

(iv) Down's syndrome associated with mosaic karyotype. Unlike the first three cytogenetic varieties of Down's syndrome which have been discussed, mosaicism must arise as a consequence of a post-zygotic non-disjunctional event. This may occur in a normal zygote, or in a trisomic zygote, and may occur in any tissue where the cells are dividing and at any stage of development. It is unlikely to give rise to a mosaic if it happens at the first cleavage division of a normal zygote, since this will give only two cell lines, 45,G– and 47,G+ and the first of these is likely to be inviable; but it may occur at any subsequent division, with the result that normal and trisomic cell lines coexist. Taylor (1968b) has shown that vigorous cell selection takes place in the stem cells of peripheral blood leucocytes in infants with such mosaicism, and that in any given child either the normal or the trisomic cell lines tend to increase in proportion; once a direction is established it seems to be maintained. This is not found in adult mosaics; presumably in them an equilibrium has been established. Since it is found in most mosaics (see above) that trisomic cells are found in greater proportion in fibroblasts than in blood cells, perhaps selection in blood cells is more commonly against trisomic cells than in their favour. This would imply that there may be a number of mosaics where the trisomy is absent

altogether in blood cells (e.g. the case of Warkany *et al.*, 1964 where no trisomic blood cells and only 17% of trisomic fibroblasts were found).

Mosaicism may also occur through non-disjunction in a trisomic zygote, with loss of a G chromosome in a cell line, which thus reverts to normal. It would seem that this is what has happened in a proportion of mosaic mothers of trisomic mongols; for example the mosaic mother of two mongol children reported by Smith *et al.* (1962), was 19 at the birth of the first mongol, and was herself born when her mother was 39 years old; Weinstein and Warkany (1963) mention a very similar case. The findings of Mikkelsen (1967), already mentioned, that the maternal grandmothers of 62 regular trisomic mongols born to young mothers were significantly older than the paternal grandmothers, suggested that there was a significant proportion of mosaicism in the mothers due to non-disjunction in a trisomic gamete.

The proportion of mosaics who arise through non-disjunction in normal and in trisomic zygotes might be guessed at by means of a study of the maternal age in mosaic mongolism, but such cases are so rare that figures for this do not seem to be available.

(v) Other karyotypic variants of Down's syndrome. Down's syndrome associated with translocations between a G chromosome and another autosome not of D or G groups, or with G ring or isochromosomes is very rare and cases are sporadic. Appearance of such cases is governed by the same considerations as apply to the appearance of autosomal anomalies following breakage and reunion, and need not be futher discussed here.

5. *Down's Syndrome and Leukaemia*

An association between Down's syndrome and leukaemia is well attested (Krivit and Good, 1957; Stewart *et al.*, 1958). Stewart *et al.* estimated that the frequency of leukaemia in mongols was 15 times that in the general population, and Holland *et al.* (1962) 18 times. Holland *et al.* found that other malignancies were 2½ times as common in mongols, but this figure, unlike that for leukaemia, was not significant. In addition to this a number of instances of mongolism and leukaemia occurring in the same families have been reported (reviewed in Penrose and Smith, 1966).

It was at one time thought that genes in chromosome 21 were involved in leucopoiesis and in leukaemia, on the grounds that, firstly, this chromosome appeared to be involved both in mongolism

and in formation of the deleted "Philadelphia" chromosome characteristically found in chronic myeloid leukaemia; and that second, alkaline phosphatase is generally increased in amount in the leucocytes of mongols and decreased in chronic myeloid leukaemia (King *et al.*, 1962). This hypothesis, always rather tenuous, has been destroyed by two facts; firstly that leukaemia of all types occurs in mongols, and chronic myeloid leukaemia is not the most common variety (acute leukaemias are common, and lymphatic leukaemia is as common as myeloid); and secondly by the demonstration by O'Riordan *et al.* (1971) that the Ph_1 chromosome is a deleted chromosome 22 and not the chromosome which is trisomic in mongols. So the reason for the very real association between Down's syndrome and leukaemia remains unexplained.

6. Meiosis in Down's Syndome

Meiotic studies are of interest in Down's syndrome since it is the only autosomal aneuploidy compatible with regular survival to reproductive age.

Nothing is known of the cytology of meiosis in female mongols, save that the small number of mongol mothers known, with their roughly 50% mongol offspring, indicate that meiosis proceeds normally, with inevitable non-disjunction producing 50% trisomic gametes, and that there is no evidence for prezygotic selection against these trisomic gametes.

Meiosis has been studied in male mongols by several authors (reviewed by Kjessler, 1970, and Hulten and Lindsten, 1970). There seems to be clear evidence that the additional G chromosome may either appear as a univalent or take part in formation of a trivalent in primary spermatocytes. Kjessler found persistent spermatogenesis in two male mongols, but as yet no case has been reported of a male mongol fathering a child. Hultén and Lindsten (1970) observed trivalents of various different morphological types in spermatocytes in mongols and carriers with D/G translocations (Chapter 3).

B. Patau's Syndrome: Typically 47,XX or XY,D(13)+

1. Introduction

This trisomic syndrome was first described by Patau *et al.* (1960). Like Edwards' syndrome (trisomy 18) which will be described next, and which, with Patau's syndrome, constitute the only two autosomal trisomies other than Down's syndrome which are compatible with post-natal life, it is invariably associated with very severe

congenital malformations which are incompatible with prolonged or useful life. As in Down's syndrome there is evidence that virtually every body tissue or system examined is in some way abnormal. In all cases studied autoradiographically it has been found that the reduplicated chromosome is No. 13 (D1) Denver (Yunis *et al.*, 1964b; Gianelli, 1965; Buchner *et al.*, 1965). Despite the severity of the malformations found in Patau's syndrome, it must be assumed, as in the case of chromosome 21 in Down's syndrome, that chromosome No. 13 must be relatively inert genetically; survival up to and after birth with trisomy for a chromosome of this size would not otherwise be possible.

2. Incidence of Patau's syndrome

Taylor (1968) has collected figures for the birth incidence, mainly from Southeast England, which show 15 cases in 114,033 births (1/7602) or about 0.13/1000. The 95% confidence limits for this figure would be roughly 0.06-0.20/1000, but not too much significance should be read into these figures as firstly it is always possible that some cases dying at birth are not ascertained, and secondly, if there is not a constant expectation of incidence (i.e. possible clustering of cases due to environmental causes, as appears possible for Down's syndrome), even a series as large as this could be misleading. However, it is certainly a rare condition, with an ascertainment rate of not more than 1/5000 births.

3. Features of Patau's Syndrome (Fig. 55)

The data are taken mainly from Taylor (1968 and 1969) and Polani (1969). Birth weight is low (mean 2610 g, 5 lb 12 oz). The head is small and abnormally shaped; eyes often small (microphthalmia) or absent (anopththalmia), with colobomata (deficiency) of the iris in a third. Half have a hare lip and three-quarters a cleft palate. The face shows hypertelorism, epicanthic folds, a large flat triangular nose, thin mouth, micrognathia (small mandible) and low set malformed ears. In three-quarters of cases the brain shows some degree of fusion of the cerebral hemispheres, with arrhinencephaly.

The most conspicuous feature in the rest of the body is polydactyly (extra digits), present in 60-80%, and sometimes abnormal external genitalia. But internally there are widespread abnormalities (Marin-Papilla *et al.*, 1964); cardiovascular anomalies in 80%, polycystic kidneys in a third; often reduplicated ureters or Mullerian ducts; excess of normal tissue in many organs such as liver, kidney, pancreas, lung, etc. Often there is *situs inversus* of the viscera

Fig. 55. Patau's syndrome. An artist's impression of an infant showing a composite of features seen in photographs published by Taylor (1968) and others; notably the large triangular nose, "old man" appearance, microphthalmia of left eye and polydactyly of both hands.

(which suggests to these authors that the condition might arise through non-disjunction in a twinning event).

Many of these abnormalities are non-specific, and are often found also (for example) in Edwards' syndrome; but one or two, such as the facial appearance, large nose, arrhinencephaly and polydactyly are rather typical of this condition. The dermatoglyphics (*q.v.*) are abnormal.

One curious biochemical feature is persistence of fetal haemoglobin at birth (Huehns *et al.*, 1964), probably attributable to delayed development. As might be expected, mental deficiency (where measurable) is profound. The infants appear deaf (and often

also blind). Fits are common. There is failure to thrive, and the expectation of life is very poor. Half are dead by the age of one month, three-quarters by six months, and four-fifths by a year. Mean survival is 89 days. Occasional cases have survived into later childhood.

4. Genesis of Patau's Syndrome

Most cases (perhaps 4/5) are standard trisomics for chromosome 13. Most of the rest have unbalanced translocations with effective trisomy 13. Most of these are of the 46,D−,t(DqDq)+ variety, but in about a quarter the translocation involves another non-acrocentric autosome. The condition does not seem to arise as a consequence of D/G translocations. Occasional cases resembling the syndrome have a ring D chromosome, or even an apparently normal karyotype.

Little is known of the aetiology. Mean maternal age is raised (31.6 years at birth of infant) but less than in Down's syndrome. Maternal age distribution is bimodal, indicating the presence of age-dependent and age-independent groups; but the maternal age-independent group seems to be larger than in Down's syndrome.

There also seems to be evidence (Taylor, 1968) for excess of winter conceptions; 79% of Taylor's series were conceived between September and February and these conceptions appear to have comprised most of the age-dependent mothers; mean maternal age for summer conceptions was 28.6, for winter 32.7. The trend for winter conceptions was found in six successive years. These very interesting findings are as yet unexplained.

Very occasional cases of the unbalanced D/D translocation variety of Patau's syndrome may arise as a consequence of a balanced translocation in a parent. But Hamerton's (1968) figures indicate that the risk to a parent carrying such a balanced translocation (which will be remembered as the commonest translocation found in the population at large, with an incidence of about 1/800) of having an affected child is probably less than 1%; the adjacent type of segregation (*q.v.*) must be very rare at meiosis. However, carriers of a balanced translocation of this type may pass it to half their offspring. Such a family, with the translocation present in three generations, but with no case of phenotypic abnormality, has been described by Krmpotic *et al.* (1968); and also two families by Palmer *et al.* (1969). In one of these families there were nine instances of a D−,t(13q14q)+ balanced translocation in three generations, and there was a history of excessive numbers of miscarriages; in the other a t(13q14q)+,t(13p14p)+ translocational combination was found in

six members from three generations. Both of these families were ascertained, interestingly, through a propositus with Down's syndrome.

It is clear that a condition comparable to a 21/21 translocation could arise in the D group chromosomes, where no normal offspring could be produced. This has not been described yet for chromosome 13 (with all living offspring having Patau's syndrome), but a case involving 15/15 centric fusion has been recorded (Lucas, 1969) associated with recurrent abortions and no viable offspring.

Mosaicism in one father of a case of Patau's syndrome has been recorded (Taylor, 1969) and this may on rare occasions be an aetiological factor.

5. Other Aspects of Patau's Syndrome

Not much is known of the incidence of Patau's syndrome in non-European races. Yu *et al.* (1970) have described four cases in Chinese infants, with an estimated incidence of 0.12/1000 (1/8605), and features generally reminiscent of those found in Caucasian infants.

Patau's syndrome with other associated abnormalities has been described. Double aneuploidy has been described (Zellweger and Abbo, 1967). France *et al.* (1967) have described an interesting case with XO/XY mosaicism coexisting. And the interesting family described by Green *et al.* (1968) has already been mentioned; a mother and two daughters had 46/47D+ mosaicism, suggesting a genetic mechanism responsible for mosaicism.

C. Edwards' Syndrome: Typically 47,XX (or XY), E(18)+

1. Introduction

This trisomic syndrome was first described by Edwards *et al.* and almost simultaneously by Patau *et al.* in 1960. Like Patau's syndrome it is always associated with very severe congenital anomalies, affecting almost ail tissues and systems, and incompatible with prolonged or useful life. Yunis *et al.* (1964a) first showed that the chromosome which is trisomic in the standard condition is No. 18 (it was earlier not possible to distinguish between 17 and 18 on morphological grounds alone) and all subsequent studies have confirmed this. As with chromosomes 13 and 21, it is likely that chromosome 18 is rather inert genetically, for otherwise trisomy would not be compatible with survival to birth and for a limited time thereafter.

2. Incidence of Edwards' Syndrome

Taylor (1969) found from collected figures (mainly from Southeast England) a total incidence of 17/115,032 births, that is, 1/6766. Conen and Erkman (1967) found 8/89,309 cases in their American series, which they interpreted (after statistical correction) as representing about 1/4500. These figures are very similar to those for Patau's syndrome and should be accorded the same significance; that the true figure may be higher, due to lack of ascertainment of some early-dying cases. There is an impression from most authors (e.g. Valentine, 1969) that Edwards' syndrome is commoner than Patau's. However, some reports have appeared recently which suggest that a very remarkable clustering of cases may sometimes occur, and that the population incidence can on occasion be much higher than that which has been given. Kardon *et al.* (1970), Shapiro (1970) and Anderson *et al.* (1970) reported an incidence of 1/600, 1/774 and 1/800 respectively over a six-month period (February-July, 1970), and this has been corroborated by Cohen (1971) who found three cases in 1570 births from March–October 1970. Cohen's figures are even more remarkable in that he found an incidence of about 1/1000 in Buffalo, U.S.A. (15 cases in 14,384 births) over a five-year period (1966-1970 inclusive) and in 1968 there were seven cases in 3137 births (about 1/450). All these cases were confirmed by cytogenetic analysis. Such figures could hardly have arisen by chance if the expectation of a trisomy 18 birth were the same as that in Southeast England, and suggest either that there was some marked genetic difference in susceptibility between the two populations or, more probably in view of the clustering within Cohen's series, that some environmental factor is responsible for "epidemics" of trisomic births. An epidemic viral infection (such as influenza) would appear to be a possible factor, in view of the suggested association between infective hepatitis and frequency of Down's syndrome (*q.v.*).

3. Features of Edwards' Syndrome (Fig. 56)

The data again come mainly from Taylor (1968, 1969) and Polani (1969). Birth weight is low (mean 2243 g, 4 lb 15 oz) and the infant looks fragile and premature. The head and facies have a characteristic appearance. The head is usually long and narrow with prominent occiput. The face is round or squarish, with wide-spaced eyes, small mouth (microstomia) and small receding jaw (micrognathia). Eye defects (especially microphthalmia) are common. The ears are low-set and usually malformed. There is often redundant

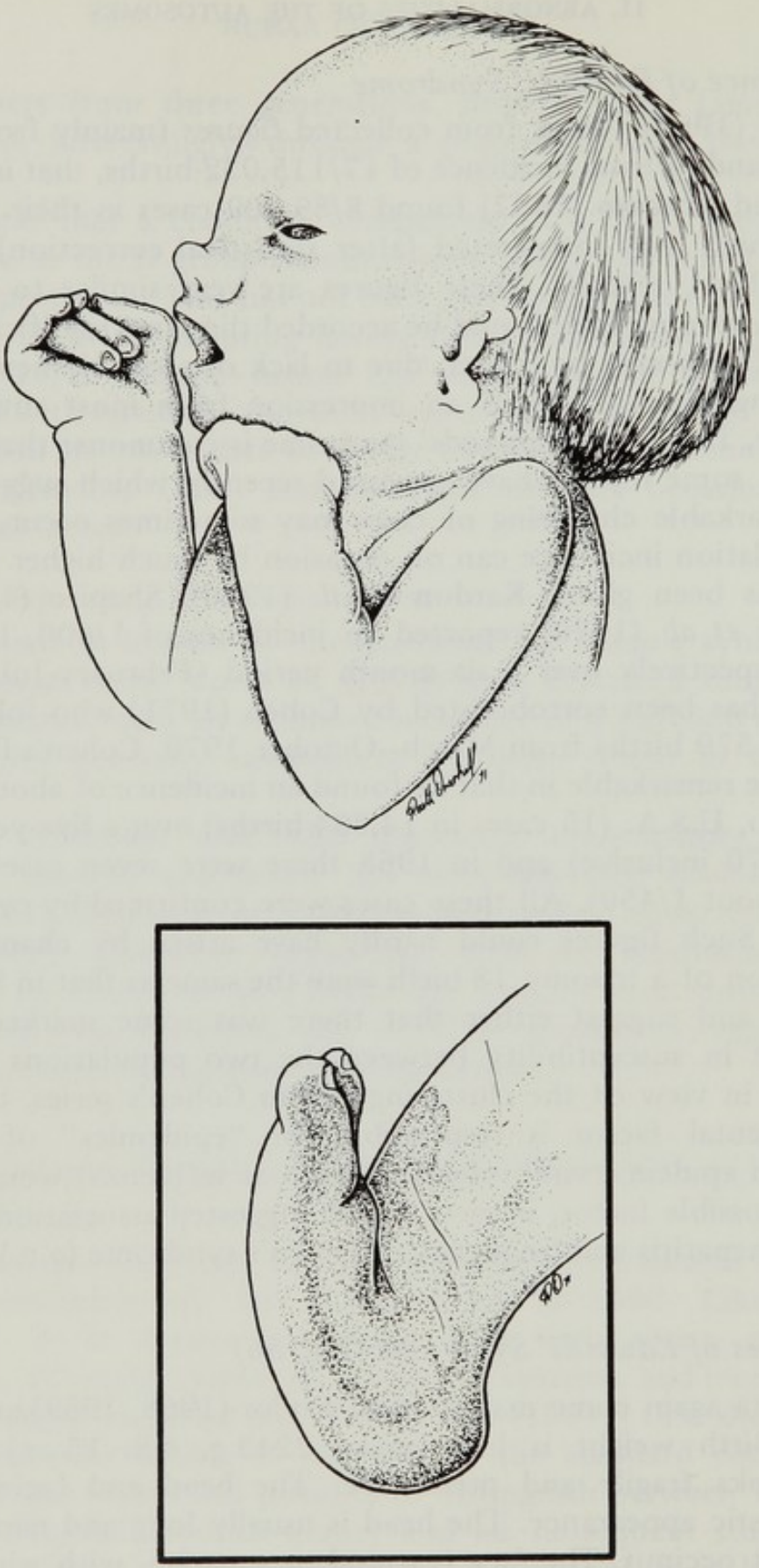

Fig. 56. Edwards' syndrome. An artist's impression of an infant showing a composite of features seen in photographs published by Taylor (1968a) and others, notably the long narrow head with prominent occiput, low-set malformed ear, and flexion deformity of the hands. The lower picture shows the appearance of a characteristic "rocker-bottom" foot.

skin on the nape of the neck, with webbing. One quarter of cases have a cleft lip or palate.

There are characteristic changes in the post-cranial skeleto-muscular system. The sternum is short and the pelvis small, but most typical is a general increase in muscle tone (not associated with enhanced reflexes, and so of uncertain neurological significance) associated with flexion deformities. These are particularly obvious in the hands, where tight flexion of the metacarpophalangeal joints is associated with axial deviation of fingers, especially the first, which overlies the others; and in the feet where there is a prominent, vertical talus, with talipes equinovalgus, producing so-called "rocker-bottom" feet with a rounded, convex sole. The big toe is short and dorsiflexed. Other mesodermally-derived tissues are affected. Cardio-vascular anomalies (such as patent interventricular septum and/or ductus arteriosus) are very common, as are renal anomalies.

Ectodermal tissues are also affected. The nails and nipples are hypoplastic, and the dermatoglyphics are abnormal (Chapter 12), the most notable feature being the presence of a large proportion of simple arches on the fingers.

Finally, umbilical and inguinal herniae are common, and a single umbilical artery is sometimes seen. The frequency of all these and other anomalies is summarized in Taylor (1969). It will be noted that quite a number of them are also found in Patau's syndrome, and presumably represent non-specific consequences of early develop-mental retardation. But the head-form, facies and the flexion deformities seem to be rather specific.

As might be expected, mental deficiency, where measurable, seems to be profound and invariable.

Expectation of life is comparable to that in Patau's syndrome, but if anything, less. One-third are dead by one month, half by two months, three-quarters by six months and 90% by one year (Polani, 1969). A very few survive, with severe defect, into later childhood.

Finally, there is a notable excess of females amongst ascertained cases (about 80%) in all series studied (113/143 in Taylor's 1968 series). This seems to be associated with greater mortality amongst afflicted males; the mean survival was found by Taylor (1968) to be 71 days; but for females (21) it was 134 days and for males (4) only 14.7 days.

4. Genesis of Edwards' Syndrome

As in Patau's syndrome, about 4/5 of cases seem to be standard trisomics. The remainder show a variety of cytogenetic variants;

Taylor (1968) found 21 trisomics among 27 cases, two double trisomics (48,XXX,18+ and 48,XXY,18+), two mosaics, one patient with a structurally abnormal chromosome 18 and one karyotypically normal. Various translocations have been reported in other series, most commonly involving the D and E group chromosomes (though B/E and E/G translocations have also been reported (Polani, 1969). Ring 18 chromosomes (*q.v.*) can produce Edwards-type phenotypes. The high incidence of double trisomies (estimated at 2-10% for double trisomies involving the sex chromosomes) is of particular interest—it could hardly arise as a consequence of two unrelated events, and therefore suggests a common genetic or environmental cause.

Little is known of the aetiology. Mean maternal age is raised (31.7 years at birth of the child in Taylor's series). As in Patau's syndrome there seems to be some excess of winter conceptions (67% between September and February) and the winter-conceiving mothers were older (31.8 years) than the summer-conceiving mothers (25.2 years) in Taylor's series. Conen and Erkman (1967) also found a seasonal incidence. But the numbers are too small to gauge the significance. However, in short, there appears to be a maternal age effect which, as in Patau's syndrome, is less pronounced than in Down's syndrome. The remarkable clustering of cases already mentioned certainly suggests that environmental causes (possible outbreaks of viral infection), may be responsible for the greatly increased incidence sometimes seen.

Rare cases of the syndrome arising through an unbalanced translocation inherited from a parent with a balanced translocation have been described. Mosaicism in a parent as a causal factor has not yet been described (Taylor, 1969) but could be a rare aetiology.

5. Other Aspects of Edwards' Syndrome

There is little data on the incidence of Edwards' syndrome in non-Europeans. Emanuel *et al.* (1970) have described four cases in Chinese infants; the general features appeared similar, and the incidence was estimated at 0.15/1000 births. A history of diagnostic radiography was recorded for two of the mothers, but the significance of this could not be assessed.

D. Other Possible Autosomal Trisomies and Aneuploidies Involving a Single Chromosome

A fair number of cases have been described in which an additional chromosome (usually small) has been found in one or more members

of a family, with or without multiple congenital anomalies. The difficulty of interpretation of these cases relates to the difficulty in identifying the chromosome involved; although a small metacentric or acrocentric chromosome may resemble a group F or G chromosome morphologically, it may in fact be a deleted chromosome from almost any other group, and the small size makes autoradiographic identification difficult or impossible. So many or most of these cases fall in the category of 47,XX or XY,?+. No regularly occurring trisomy with constant phenotypic features has been reported other than the three already described; the only other possible candidate is 47,XX or XY,22+. Polani (1964) reviewed 11 possible cases but found lack of unifying clinical features, and later it was found that one or two were 47,XYY, or mosaics (Polani, 1969).

However, some authors feel that there is recognizable trisomy 22 syndrome. For instance, Uchida *et al.* (1968) described two siblings with a mosaic mother, with abnormalities, but not with Down's syndrome. The brother and sister had epicanthus, low-set floppy ears, micrognathia and other abnormalities. The sister, who died at 20½ months, had a cleft palate and an atrial septal defect. The brother, who died at 48 hours, had cardiovascular anomalies. The first diagnosis was partial trisomy 18, but a number of resemblances were noticed to a case described by Ishmael and Laurence (1965), and trisomy 22 was considered a possibility. These cases also resemble the two sisters described by Gustavson *et al.* (1962) who also had mental deficiency, microcephaly, cleft palate, micrognathia and low-set malformed ears associated with an extra small autosome morphologically similar to a chromosome of the G group. Fluorescence studies in similar cases in the future should reveal whether the autosome in question is in fact No. 22 or whether it is a partially deleted chromosome from another group, such as a No. 18.

Extra small metacentric chromosomes have been described by a number of authors; sometimes these occur in a number of members of a family without any obvious phenotypic effects (e.g. the cases reported by Smith *et al.* (1965), Ridler *et al.* (1970), Hoehn *et al.* (1970) and Armendares *et al.* (1969)). In some instances these may be deleted X chromosomes (especially when occurring in phenotypically normal women as in Ridler *et al.*'s family); in others they may represent the consequence of a translocation. An extra small metacentric chromosome with satellites at both ends has been described on at least three occasions (Ellis *et al.*, 1962; Mukherjee and Burdette, 1966; Armendares *et al.*, 1969). Possible origin has been discussed by Ellis *et al.* (1962); the chromosome might

represent a reciprocal translocation product, or an isochromosome 21 (especially if mongoloid features are present) or be produced in some other way. Sometimes small extra metacentrics are associated with multiple anomalies, as in the case of Fröland *et al.* (1963), Ishmael and Laurence (1968) and Mukherjee *et al.* (1968), in which case they probably represent deleted autosomes, most probably 17 or 18.

Other autosomal trisomies than 13, 18 and 21 are, of course, found in spontaneously aborted embryos (Chapter 9). One case is known of trisomy 16 surviving after birth, with multiple anomalies (Taylor, 1969). Arakaki and Waxman (1969) have described a father of a trisomy 16 aborted fetus who was himself a mosaic for the trisomy. And cases of normal/trisomy C and normal/trisomy F mosaicism have been described (Lawler *et al.*, 1966; Gagnon *et al.*, 1963; Mosonyi *et al.*, 1966).

E. Autosomal Monosomy

For some years after human chromosome studies began it was thought that all monosomies for an autosome were lethal, probably in the early embryonic period. However, there is one possible exception, monosomy G (probably 21). A syndrome was first described by Lejeune *et al.* in 1964 in association with a deletion of a G group chromosome, and given the name "anti-mongolism". Cases with similar features have since been described in which there is apparently complete monosomy for a G chromosome (e.g. Thorburn and Johnson, 1966; Al-Aish *et al.*, 1967; Hall *et al.*, 1967); of course latent mosaicism cannot be excluded in such cases, but the most careful studies have failed to reveal it. A similar case with a ring G chromosome has also been described (Hoefnagel *et al.*, 1967).

The features of anti-mongolism are as follows (Taylor, 1969): low birth weight, failure to thrive, mental retardation, and multiple somatic anomalies, including a characteristic facies (rather opposite to that of Down's syndrome, in that there is a narrow face with a beaky nose, eyes with anti-mongoloid slant, micrognathia and low-set ears) and various skeletal anomalies, cardiac defects and pyloric stenosis. Increased muscle tone is usual.

There is no evidence of increased maternal age, and all cases seem to be sporadic; presumably they arise through either somatic non-disjunction or following chromosome breakage (according to the cytogenetic type) and it would not therefore be expected that there would be any common aetiology.

F. Triploidy

Triploidy in man is a very rare condition (except in abortuses) and seems to be incompatible with survival to birth unless it exists in mosaic form, with a diploid cell line also present (Polani, 1969). The first such case was described by Böök and Santesson (1960). Full triploidy has been described by Edwards *et al.* (1967), in a 32-week abortus, and Bernard *et al.* (1967). Schindler and Mikamo (1970) have reviewed 10 cases, of which seven were diploid/triploid mosaics. All showed multiple anomalies. In two cases there was hemiatrophy (or hemi-hypertrophy). Syndactyly was found in six, from both groups.

Most triploids described are 69,XXY. Polani (1969) found twenty XXY, nine XXX and two XYY cases described in the literature. The XXY triploids have usually been found to be chromatin-negative, and intersexuality is common. Mental defect seems to be the rule in those who survive long enough to be studied.

Triploidy must arise either from a diploid ovum (digyny) or double fertilization (or, just possibly, a diploid sperm, diandry). It is not known which event is commonest, since the condition is so rare.

Schmid and Vischer (1967) have described a malformed boy with 48,XXYY/71,XXXYY mosaicism.

Triploidy is a fascinating biological phenomenon. Perhaps what is most surprising is that it is compatible with cell survival at all; each affected cell has 50% more genetic material than is normal. Not surprisingly, affected cells and nuclei are larger than normal (this perhaps contributes to the occasional hemihypertrophy which has been noted) but seem to divide and function like normal cells. In some animals triploidy seems to be compatible with almost complete normality (e.g. in the fowl—Comings and Okada, 1971). This is presumably because the genetic material is balanced, although increased, and it is of interest in this context that in human triploidy a second X chromosome seems usually not to be inactivated-presumably it is necessary that it remain active in order to maintain the genetic "balance" (whatever the precise significance of this may be).

In mammals generally, triploidy seems to be a rare phenomenon, and not usually compatible with phenotypic normality, unlike the state of affairs in amphibia and birds. Perhaps this relates partly to the condition of the sex chromosomes in mammals. To maintain complete genetic "balance" a triploid cell should have 1½ active X

chromosomes (or Y chromosomes) and this is not functionally possible.

III. STRUCTURAL ALTERATIONS OF AUTOSOMES

Examples have been found in man of all the varieties of structural alteration described in Chapter 8; deletions, duplications, ring chromosome formation, translocations (interchanges) of various sorts, inversions and isochromosomes. While these will be dealt with under their respective headings, there is overlap between some of the categories; thus ring chromosomes may be expressed phenotypically as a mixture of deletion and duplication of the chromosome concerned.

All these alterations occur in the population sporadically, and are therefore rare. Therefore not many distinct syndromes have yet been described in association with specific alterations. It is likely that as more cases are collected, further syndromes will be described; but the limitation to this process is the fact that every case is unique; no two deletions, for example, even where they affect the same region of the same chromosome, are likely to be identical in extent; and they will certainly not be identical in respect of the material deleted, since each individual is genetically unique. So, just as in the trisomies, but to a more marked extent, each so-called syndrome is a spectrum of phenomena, with more or less typical cases in the middle, and with less typical (sometimes unrecognizable) phenotypes at each end.

A. Deletion Syndromes

Six deletions have been recognized in the human karyotype which are associated with more or less distinctive syndromes in the majority of cases. One (21q–) has already been discussed in association with monosomy 21, as a cause of "anti-mongolism". The others are 4p–, 5p–, Dq–, 18p– and 18q– (Taylor, 1969).

In addition to these is the deletion of the long arm of chromosome 22 (giving the Philadelphia or "Ph_1" chromosome) which is found in the affected cells in chronic myeloid leukaemia; this will be discussed in Chapter 13 under "chromosomes and cancer".

1. The 5p– Syndrome; the Cri-du-chat or Cat-cry Syndrome

This, the first autosomal deletion to be recognized, was first described by Lejeune *et al.* (1963). Since then over 100 cases have

been reported (Polani, 1969). The chromosome involved was identified autoradiographically as No. 5 by German *et al.* (1964). Chromosomes 4 and 5 are very similar morphologically, but autoradiographically sort themselves into two pairs. As Miller (1970) has pointed out, there is always a danger of circular reasoning in identification, for if one member of each pair was an early and one a late replicator, the pairs could not be distinguished by this technique. However, careful measurement (Warburton *et al.*, 1969) indicates that the deleted chromosome is a member of the shorter pair, and fluorescent techniques have now confirmed the identification of the two pairs.

(a) Incidence of the cat-cry syndrome. An accurate estimate of the incidence is not yet possible because of the great rarity of the syndrome. Polani (1969) estimated it at between 1/50,000 and 1/100,000 births. Taylor (1969) found no case in 165,000 live births. But as with other rare conditions, lack of ascertainment is a major difficulty (especially as the condition is not remarkably abnormal in phenotype) and it is likely as time goes on that estimates of incidence will be revised upward.

(b) Features of the cat-cry syndrome. The most characteristic feature, and the one which first led to recognition of the syndrome is, as the name implies, the curious, faint, mewing cry of affected babies, which seems to be absolutely diagnostic. There are no reports as to whether this is due to physical or neurological abnormality.

The affected babies are small. The average birth weight (Taylor, 1969) is 2680 g (5 lb 14 oz). They fail to thrive; suffer from considerable mental defect; usually have hypotonic muscles; and have a number of abnormalities in the cranial region, but not many post-cranially, except for cardiovascular anomalies, which are not uncommon.

The head typically shows some microcephaly, epicanthic folds, anti-mongoloid slant to the palpebral fissure, strabismus, large prominent cornea, low-set ears, and a rounded moon face with hypertelorism. However, despite this formidable-sounding list, these are features which might be found in any group of mental defectives, and there is nothing very distinctive in the features of published cases which would immediately suggest a diagnosis.

About two-thirds of ascertained cases are females (Polani, 1969; Taylor, 1969) and it is not known whether this represents the incidence at birth or is due to differential mortality (such as is found in Edwards' syndrome).

The condition is quite compatible with survival; only one of 23 cases ascertained by Taylor (1969) had died, and the eldest were

surviving into their teems at the time of publication, with psychomotor retardation.

(c) Genesis of the 5p– syndrome. Little is known of the aetiology. Polani (1969) estimates that 1/6 of cases are due to interchanges involving chromosome 5; 5/C, 5/D and 5/G translocations have all been described. Mosaicism in a mother with a cri-du-chat child has been described (Philip *et al.*, 1970). The condition has been described in association with a ring-B chromosome (e.g. Steele *et al.*, 1966). But the great majority of cases (including the last two categories) must be due to sporadic chromosome breakage with unascertainable cause. This is borne out by the absence of any parental age influence (Taylor, 1969; Polani, 1969).

2. The 4p– Syndrome

Some cases with deletion of a B-group chromosome seem to show rather different features from the classical cat-cry case, and are now thought, although excessively rare, to show deletion of the short arm of chromosome No. 4. The syndrome was first described by Sidbury *et al.* (1964), and autoradiographic identification of the chromosome involved as No. 4 was first made by Wolf *et al.* (1965). The syndrome is sometimes called the Wolf-Hirschhorn syndrome. Polani (1969) estimated that one third of B-deletion cases might be 4p–, but Wilson *et al.* (1970) reviewing the condition only found reports of 12 cases. Wilcock *et al* (1970) have given a detailed description of a case, with a review of five cases.

The incidence of the 4p– syndrome is not yet possible to estimate, but it is clearly very low. Taylor (1969) found one case in 165,000 births. The characteristic features of the condition seem to be as follows: there is no cat-cry; and there are rather more profound defects generally than in the 5p– syndrome, including especially midline defects, some of them reminiscent of Patau's syndrome. There may be hypertelorism and broad nose-root, scalp skin dysplasia and cleft palate (both present in 4/4 of Wilcock *et al.'s* series), abnormalities of iris (colobomata) and lens, micrognathia, hydrocephalus, and various skeletal defects. Hypospadias is found in boys. The condition is more lethal than the 5p– syndrome, but survival into the teens has been reported (Taylor, 1969), with the inevitable profound psychomotor retardation.

Virtually nothing is known of the aetiology, but it may reasonably be guessed that most cases are sporadic. A case with midline defects and hypospadias associated with a probable ring-4 chromosome (not confirmed autoradiographically) has been described by Carter *et al.* (1969).

3. 46,XX or XY, Dq– Syndrome

Taylor (1969) has reviewed three possible cases of this syndrome, originally described by Laurent *et al.* (1967). The propositi showed Patau-like features, with Patau facies (but without hare lip or cleft palate), microphthalmia and congenital heart disease; the longest survival was 3½ months. It is perhaps premature to generalize as to whether this constitutes a distinctive syndrome on the evidence of only three cases.

4. 46,XX or XY, 18p– Syndrome

Deletion of the short arm of chromosome 18 (which may be total) was first described by de Grouchy *et al.* (1963). De Grouchy (1969) reviewed the findings of 23 cases, and Fischer *et al.* (1970) report a total of 28 cases in the literature. Gianelli and Howlett identified the chromosome involved as No. 18 in two cases (Polani, 1969). The incidence of the condition has not been estimated, but it is both sporadic and rare.

Features of the condition are mental deficiency, ocular anomalies (hypertelorism, epicanthic folds, ptosis, squint) in all cases (Polani, 1969), flat nose-bridge, small jaw and low-set ears. One third are described as having the Bonnevie-Ullrich syndrome. But the somatic anomalies are relatively minor (although occasionally severe brain anomalies have been found) and survival is usual, the oldest case recorded being in the thirties. Fischer *et al.* (1970) and Stewart *et al.* (1970) have described cases with absence of IgA in the serum, but this is not a general finding and the significance is not yet apparent.

All cases so far described have been sporadic except for one instance where a mentally defective mother with the deletion had two affected children (Uchida *et al.*, 1965) by different fathers; one of the children had arrhinencephaly. Maternal age is raised (mean 34.2; Polani, 1969).

5. 46,XX or XY, 18q– Syndrome

This was first described by de Grouchy *et al.* (1964). De Grouchy (1969) reviewed 24 cases, which seem to have a number of constant features. One-quarter to one-third of the long arm of chromosome No. 18 was deleted. The incidence is not known. Characteristic features seem to be: mental defect of some degree with microcephaly; naso-maxillary dysplasia, so that the forehead and lower jaw appear prominent while the centre of the face appears sunken. The eyes appear deep-set, the mouth has been described as carp-like, and there may be dermal tags on the cheeks. The ears have prominent helix and antihelix, but the external auditory meatus is narrowed or

closed. The fingers are long and tapering and there are abnormal dermatoglyphics. Congenital heart anomalies are found in one-third. Survival is usual.

All cases so far described have been sporadic except for one case with an unbalanced 18/G translocation (Law and Masterson, 1966) inherited from a mother with a balanced translocation. Maternal age is slightly raised (29.8 in 11 cases; Taylor, 1969) but this may not be significant.

6. Other Autosomal Deletion Syndromes

Other possible autosomal deletion syndromes have been reviewed by Polani (1969), but there do not at present seem to be clear-cut instances.

B. Duplications

Although quite a lot of cases have been described in which there appears to be extra material present on an autosomal arm, in association with various congenital anomalities; it has not yet been found possible with present techniques to distinguish between a possible duplication and an unbalanced translocation. This may become possible in the future with fluorescent or other techniques, but at present there is nothing further that can usefully be said, and no regular syndromes due to simple autosomal duplication have been described, although there is no reason to suppose that they may not exist.

C. Ring Autosomes

Ring chromosomes from most of the autosomal groups have been recorded (reviewed in Polani, 1969); 1,B, C, D, E, and Gr are all known. Ring chromosomes evolve during repeated cell divisions, as explained in Chapter 8, into further rings containing various combinations of duplications and deficiencies for the chromosome concerned. The symptomatology will depend, therefore, on the stage of development at which the ring has appeared, and how many cells and tissues are involved; and on a series of random events occurring during successive cell divisions. In most phenotypically affected individuals the ring has presumably appeared at a fairly early stage of development, and most tissues and organs contain it.

Individuals with ring D, E, and G chromosomes have been described who show various phenotypic abnormalities ranging from

those characteristic of deletions to those characteristic of trisomies for the chromosome concerned, or a mixture of both. The phenotypes of individuals with ring chromosomes in other groups (in which trisomy and deletion are not familiar) are rather inconstant (Polani, 1969).

The cytological behaviour of ring chromosomes is of considerable interest; studies have been published by Cooke and Gordon (1965), Gripenberg (1967) and Kistenmacher and Punnett (1970) on the behaviour of human ring chromosomes. As might be expected, there seems to be considerable variation between different ring chromosomes as to the appearance of interlocked rings, double-sized rings, coiling abnormalities, dicentrics and so on.

D. Inversions

As noted in Chapter 8, inversions may be paracentric or pericentric, but paracentric inversions in man have not been described because they produce no change in mitotic chromosome morphology with ordinary staining methods (it is possible that fluorescent staining could detect them) and would only be detected through meiotic studies. Pericentric inversions do occur and have been described in man. Their incidence is not known—the only study on an adequate number of individuals is that of Waltzer *et al.* (1969) who found three instances in 2400 individuals. As only the most obvious are likely to be detected with regularity, the true frequency is likely to be a good deal higher.

An inversion will have no effect on the phenotype of the individual carrying it, since the total amount of genetic material present remains unaltered. The only danger which individuals with inversions risk is that an unbalanced gamete may be produced at meiosis. It has long been known from studies on plants and *Drosophila* that a chromosome with an inversion pairs with its homologue at first meiotic prophase by the formation of an inversion loop (Fig. 47f). By this means pairing of homologous regions of the two chromosomes is made possible. Provided no crossing-over takes place in the inversion loop, segregation of the two homologues at the first meiotic division proceeds normally. However, if crossing-over should take place in the loop, the two cross-over chromatids will be abnormal, as shown in Fig. 47. The four gametes produced will comprise one normal, one carrying the inversion, and two which have a combination of duplication and deficiency. These could form a zygote which was either so abnormal that it was aborted, or

developed into an abnormal individual. If the inversion is pericentric, all the chromatids produced will be monocentric, but if it is paracentric, crossing-over in the loop will of course result in the formation of dicentric and acentric chromatids.

A number of families are known in which a pericentric inversion is present in several members. Jacobs *et al.* (1967) described three families with pericentric inversions of a C group chromosome. There was no evidence of increased numbers of spontaneous abortions, and no abnormal individuals. Westkamp *et al.* (1969) described a family in which 17 members carried a pericentric inversion of a No. 2 chromosome, with no abnormals. Crandall and Sparkes (1970) described pericentric inversion of a No. 15 chromosome in nine members of a family with no abnormals. Wilson *et al.* (1970) described a family with a pericentric inversion of chromosome No. 4, ascertained through an unbalanced propositus. The mother and seven relatives carried the inversion. This last family is almost the only one in the literature in which an unbalanced individual has been born, and it is evident that the risk to an individual carrying such an inversion of having an abnormal child is very low; either crossing-over in the inversion loop is unusual (though this is surprising in pericentric inversions generally, since they must involve a considerable proportion of the length of a chromosome to be detected at all) or the zygote is so abnormal that it fails to implant or aborts very early.

Pericentric inversion can produce good marker chromosomes which can be used in linkage studies (as in the paper by Westkamp *et al.* quoted above) or in tracing family relationships (as in one of the instances described in the paper by Jacobs *et al.* (1967) where the inversion was found in a father and son, and also in another apparently unrelated boy who proved to be an illegitimate child of the same father. The main clinical effect of inversions may be to produce reduced fertility, because of the unbalanced gametes which may be produced.

E. Shifts

As mentioned in Chapter 8, these have not yet been described in man.

F. Interchanges: Simple Translocations, Insertions, etc.

Interchanges involving two (or sometimes more) chromosomes are perhaps the commonest structural alterations seen in human chromo-

somes. Indeed the first phenotypic abnormality due to a chromosomal change which was described in man was due to a translocation (Turpin *et al.*, 1959). Court Brown (1967) estimated that a minimum of 0.5% of the population (and almost certainly more than this in fact) were structurally heterozygous for a chromosome pair, and the majority of this heterozygosity is probably due to translocations.

1. Reciprocal translocations

These may be, as pointed out in Chapter 8, of two types; the first, where following breakage in two separate chromosomes the acentric portions are exchanged, and the second where the two centric fragments fuse and the acentric fragments also fuse, to give a dicentric chromosome and an acentric fragment. This second type will not be further discussed here, since the acentric fragment is likely to be lost when the cell next divides: and if the translocation is present in the gonad the presence of an abnormal dicentric chromosome is almost certain to lead to infertility.

The type of translocation where two chromosomes exchange acentric fragments (the so-called "Robertsonian" translocation may be regarded as a special case of reciprocal translocation, although, as mentioned in Chapter 8, these may be true centric fusions with a slightly different mechanism) are of considerable genetic importance because although they will not give rise to any phenotypic effect in the individuals in whom they arise (the total amount of genetic material remains unaltered, and the translocation is referred to as "balanced"), they can give rise to abnormal ("unbalanced") gametes during the meiotic cell divisions, and hence to abnormal progeny which may either be aborted or carried to term as a phenotypically abnormal child.

The meiotic consequences of reciprocal translocations of this sort have been reviewed by C. E. Ford and Clegg (1969). The cytogenetics of meiosis is well known from studies on plant chromosomes by McClintock (1945) and others, and is summarized in Fig. 48. At meiosis the homologous portions of the four abnormal chromosomes characteristically pair up to form a ring-of-four configuration. The types of gamete which will then be formed will depend firstly on how the four chromosomes segregate into pairs at the first meiotic division; three types of segregation are possible (if non-disjunction is excluded) which have been called alternate, adjacent-1 and adjacent-2; and secondly on whether crossing-over occurs in the interstitial segments (the parts of the chromosomes between the centromeres and the position of the breakage where the interchange

has taken place). Alternate segregation will give one normal gamete, and one carrying the balanced translocation; the two types of adjacent segregation will each give rise to gametes with a mixture of duplication and deficiency for some parts of the chromosomes. With crossing-over in the interstitial segment, the alternate and adjacent-1 types of segregation become geometrically indistinguishable, while the adjacent-2 type can give rise to four more sorts of unbalanced gamete. In short, of ten possible types of gamete which may be produced, only two are balanced, one of which carries the original translocation. It might be expected from this *a priori* that carriers of such a translocation would have normal gametes and balanced translocation gametes in equal numbers, plus an unknown proportion of unbalanced gametes.

C. E. Ford and Clegg (1969) studied 120 cases of individuals with unbalanced karyotypes, where the karyotypes of both parents were known. In 65 the translocation was inherited from the mother; in 28 from the father; and in 36 both parents were karyotypically normal; in six of these latter cases the propositus was a mosaic, indicating almost certain *de novo* translocation in the propositi themselves. Ford inferred that each translocation was transmitted, on average, through three generations before elimination.

Ford's findings on the genetics of these translocations are summarized in Table 8. It will be seen that the number of translocation heterozygotes are considerably in excess of normals, apparently contrary to the 1:1 expectation; but this may be explained by the very considerable bias introduced by the fact that all the families were ascertained because of abnormal offspring. Of interest in relation to the production of abnormal gametes is the finding that the frequency of spontaneous abortions was not much above the level for the general population. Lejeune *et al.* (1970), in a comparable study of the genetics of human reciprocal translocations, estimate that a carrier mother has a risk of 10-20% of having an unbalanced child; the risk to a carrier father is probably less, but the trend is less pronounced than with the centric fusion type of translocation.

However, as in all types of interchange, every example is different and it will never be safe to generalize about the outcome in a particular mating. Ideally advice would be given on the basis of accurate diagnosis as to the nature of the translocation and attempts to study its meiotic behaviour [as for example in the case studied by Fredga and Rayner (1967) and Fredga (1968)], knowledge of its segregation in the family concerned, and general knowledge of the

Table 8*

Sex of heterozygous parent	No. of cases	Livebirth; normal phenotype			Stillbirth or live birth with abnormal phenotype	Spontaneous abortions
		Translocation heterozygotes	Normal karyotype	Chromosomes not studied		
Male	55	53	30	7	50	33
Female	106	79	56	43	121	89
Total	161	132	86	50	171	122

* C. E. Ford and Clegg, 1969.

behaviour of human translocations. The facilities and the skills needed to do this are not yet widely available.

Collected information on 55 human reciprocal translocations has been given by Hamerton (1968) and is also tabulated in Taylor (1969). Hamerton has studied the chromosome groups involved in these translocations and related the frequency with which they are involved to the proportion of total chromosome length in the karyotype, and finds that the D and G group chromosomes are significantly more often involved than might be expected (excluding Robertsonian translocations) while some groups are significantly less often involved than might be predicted on grounds of length (group A and probably F). This probably relates to the position of chromosomes in the nucleus; the association of the acrocentrics through their common association with the nucleolus means that if they are broken, interchanges can easily occur.

The empirical genetics of the centric fusion type of translocation has already been considered under Down's syndrome. A diagram of meiotic segregation for this type of translocation, comparable to that for reciprocal translocations, is shown in Fig. 49. Empirical data indicate that the alternate type of segregation probably occurs more commonly than the adjacent types, since the expectation of unbalanced offspring is relatively low. In the D/G translocations, the adjacent-1 segregation has not yet been reported unbalanced progeny of the trisomy 13 variety have not been described (but, of course, as indicated earlier, chromosome 13 is seldom involved: usually it is chromosome 14 or 15, and trisomy of either of these may result in an inviable zygote); but the adjacent-2 variety is responsible for the unbalanced progeny of trisomy 21 type who do occur; and the figures suggest that this type of segregation is more common in the ovary than the testis. It would be of interest to know the origin of the centromere in these translocation chromosomes, since this might affect the type of segregation which occurs. Fluorescent staining of chromosomes should make this possible in a number of cases (O'Riordan *et al.*, 1971).

G. More Complex Translocations; Insertions

Translocations involving more than two breakages, such as insertions, where a segment of one chromosome is inserted into a breakage point in another, may be very difficult to diagnose in man on the basis of mitotic chromosome morphology alone, or even with the benefit of meiotic studies as well. Few such cases have therefore

been described in the literature so far. Lejeune and Berger (1965) were the first to draw attention to one particular aspect of such interchanges, the fact that two individuals in a family might have apparently identical chromosomes morphologically, but that some of these chromosomes might be genetically unbalanced. They called this "*aneusomie de recombinaison*" (aneusomy by recombination). Figure 50 (after Lejeune and Berger) shows how this situation might come about. Following formation of a quadrivalent at meiosis, and crossing-over taking place, four types of gamete may be formed which are respectively normal, balanced translocation, and two unbalanced gametes which can, under the right conditions, be indistinguishable from the other two in their chromosome morphology. De Grouchy (1965) and de Grouchy *et al.* (1966) have described further examples of "*aneusomie de recombinaison*".

Fredga and Hall (1970) have described another type of complex familial translocation, involving chromosomes 5, 9 and 13, and well supported by meiotic studies (which are essential in the proof of such complex translocations); the proper diagnosis of such complex cases may be regarded as the ultimate in cytogenetic skill, involving as it does the skilful employment of almost every important technique of human cytogenetics.

However, although these complex translocations are of great theoretical interest they are so rare, and the meiotic complexities are potentially so great, that the subject will not be further pursued.

H. Isochromosome formation

Little is known of isochromosome formation in autosomes, although it probably occurs sometimes, and may be responsible for some reported cases of D/D and G/G translocations where homologues are involved (Polani, 1969); it has also been suggested that some small extra metacentric autosomes with satellites on both arms may be isochromosomes for the short arms of a chromosome 21 (or 22).

IV. MOSAICS AND CHIMAERAS

The general question of mosaicism has been discussed under Down's syndrome where, as already stated, it may be present in some 2½% of affected individuals. Outside Down's syndrome it is much rarer. A few cases of mosaicism for other trisomies have been

described (see above). La Marche *et al.* (1967) have described a longitudinal study on a child who was a 46,XX/47,XX,17+ or 18+ mosaic, and who was found to have 90% of trisomic cells at birth. In five later cultures at ages between 10 and 16 months, only normal cells were found. Presumably selection in this direction may be expected when a really deleterious trisomy is present, but the basic genetic damage will already have been done before birth.

A mosaic mongol has been described who was one of a pair of monozygotic twins, and whose brother was normal (Lejeune *et al.*, 1962).

V. OTHER CONDITIONS POSSIBLY ASSOCIATED WITH AUTOSOMAL ABNORMALITIES

In the early period of human cytogenetic studies a number of conditions were described in which chromosomal abnormalities appeared to be sometimes present. None of these have received consistent support from later investigators. Kallén and Levan (1962) suggested that Marfan's syndrome might be associated with elongated satellites on the G chromosomes. However, when the frequency of elongated satellites in the general population was studied, it became apparent that although the relationship in the cases studied was genuine, it was unlikely to be causal. Patau *et al.* (1963) suggested that Sturge-Weber syndrome might be due to partial trisomy of an autosome; but this was not morphologically visible in most of the cases, and has failed to find confirmation. Oro-facial-digital syndrome and Stein-Leventhal syndrome have also been put forward as possibly caused by chromosomal anomalies, but Yunis (1966) was unable to confirm these findings.

Another syndrome which many workers have considered to be likely to be caused by a chromosomal abnormality is the de Lange syndrome. However, although chromosomal abnormalities have been found in a surprisingly high number of cases of this syndrome, they seem to be quite inconstant in nature, and do not afford any support for the hypothesis that the syndrome is due to a chromosomal abnormality.

Syndromes possibly associated with increased chromosomal fragility will be considered separately, as they fall into a rather different category of possible causal relationship.

§12§

Skin, Brain and the Chromosomes

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I. INTRODUCTION

Chromosomal abnormalities can and sometimes do render almost all the cells and tissues in the body abnormal to a greater or lesser degree. However, the abnormalities, whether biochemical, morphological or functional, are not easy to detect at the cytological or histological level; their obvious manifestations are apparent to the observer on a grosser scale. The two regions where abnormalities can most readily be detected are the skin and the brain; the first because there are visible abnormalities of one kind or another associated with almost every chromosomal aberration (certainly in statistical terms, if not always in a particular affected individual); and the second because even minor aberrations in development will manifest themselves in later life as an obvious and quantifiable functional deficiency.

It is not intended here to give a very detailed or critical review of either dermatoglyphic or mental abnormality in chromosome disorders, but merely to give a straightforward account of the main dermatoglyphic findings (since no work on human chromosomes would be complete without some discussion of the subject) and to comment on their possible significance and/or use; and to consider briefly the relationship between chromosome aberration and mental defect.

It is worth noting at this point that skin and brain are both derived from the embryonic ectoderm; ectodermal derivatives in vertebrates

are in general more complex than are mesodermal derivatives, both in development and in final form, and thus their development is more readily upset by anything which interferes with the normal rhythm of growth. They are therefore sensitive indicators of any early developmental abnormality, particularly in the first few months after conception.

II. DERMATOGLYPHICS

A. Introduction, Nomenclature and Methods

The study of patterns visible on the skin of the hand and fingers (and also the foot) has been pursued since the last century when Galton showed how fingerprint patterns might be classified and codified for the purpose of criminal identification. The palms of the hands and the soles of the feet display a particularly intricate pattern of epidermal ridges, associated with the openings of the ducts of the sweat glands, and considered to be highly developed in the primates as a group as an aid to prehension in their present or past arboreal existence. Of course intricate patterns on the skin surface are not confined to these regions, but they have not been so intensively studied in other regions of the body (although it is likely that there are good dividends awaiting any research worker who will study abnormalities of the whole body surface in chromosomal disorders), and will not be further considered here.

The arrangements of the dermal ridges on hand and foot may be studied by means of a hand lens, or more conveniently by making finger and hand prints which can be studied at leisure. This may be done by taking fingerprinting ink, as used by the police, rolling it out thinly on a smooth surface such as a tile, and making impressions on paper, a procedure which is not difficult with a little practice providing that the patient is cooperative and does not suffer from flexion deformities (as, for example, in Edwards' syndrome). If a three-dimensional replica is required for research purposes a cast can be taken using latex or a similar material.

It is then conventional to study the prints in the following ways:

1. Fingerprints

The patterns of the ridges on the fingertips are classified as either *arches, loops,* or *whorls* (Fig. 57). These may be differentiated by the number of *triradii* present. A triradius is a point where three ridges

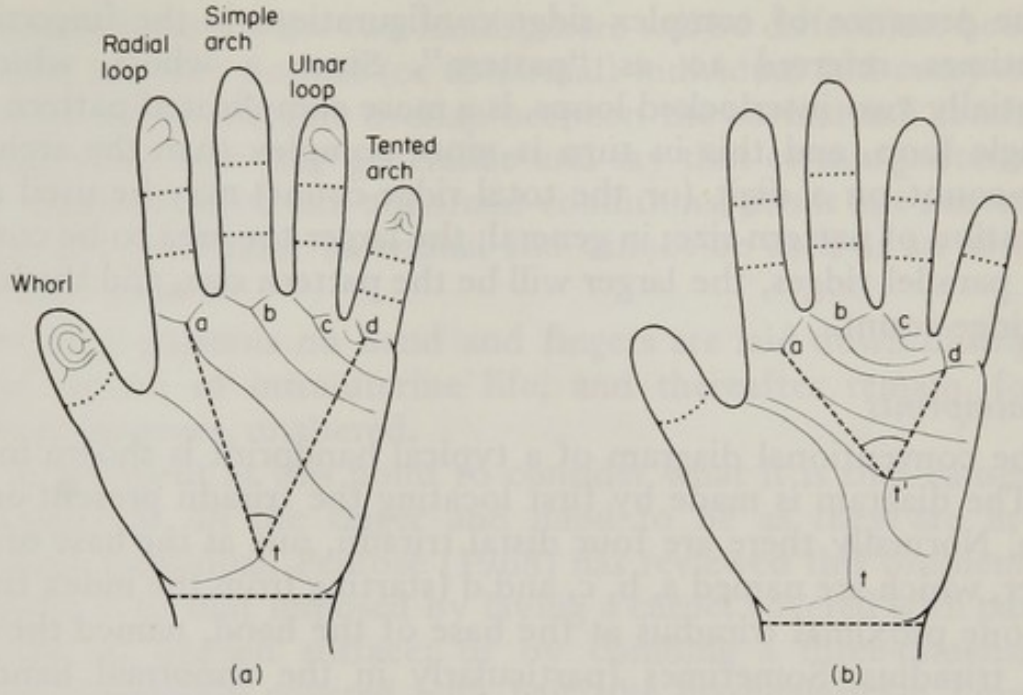

Fig. 57. (a) Diagram of the palmar surface of a hand, showing the main triradii and the epidermal ridges running from them; also the classification of the configurations seen on the pads of the fingers. a-d are the triradii at the bases of the fingers, and t the proximal palmar triradius. The atd angle is shown. (b) Rough diagram of the palmar surface of the hand of a subject with Down's syndrome; note the presence of an extra, distal palmar triradius (t'), and the consequently more obtuse atd angle.

meet (or which they approach, but end before they meet, so that there may be a single raised point at the triradius). In theory if the three ridges meet at equal angles, there will be an angle of 120° between each pair. In practice a triradius is not defined as such unless each of the three angles is at least 90° (Penrose and Smith, 1966). An arch has no associated triradius; a loop has one; and a whorl has two. Loops are further differentiated into *radial* and *ulnar* according to whether the open end of the loop faces towards the radial (thumb) or ulnar side of the hand.

The *ridge-count* has long been used in the classification of fingerprints and more recently in demonstrating similarities and differences between hands of members of families (Holt, 1961). The ridge-count of a particular fingertip is made by drawing a line from the triradius or triradii, if present, to the central point of the whorl or loop concerned and counting the number of ridges crossed *en route*. Since there are two such lines for a whorl, one for a loop and none for an arch, the total finger ridge-count will obviously be closely related to the proportions of these configurations present on the ten fingertips; the more arches and the less whorls, the lower the ridge count.

The presence of complex ridge configurations in the fingertips is sometimes referred to as "pattern". Since a whorl, which is essentially two interlocked loops, is a more complicated pattern than a single loop, and this in turn is more complex than the arch, the ridge-count on a digit (or the total ridge-count) may be used as an indication of pattern size; in general, the larger the area to be covered with parallel ridges, the larger will be the pattern size, and the higher the ridge-count.

2. Palmprints

The conventional diagram of a typical handprint is shown in Fig. 57. The diagram is made by first locating the triradii present on the palm. Normally there are four distal triradii, one at the base of each finger, which are named a, b, c, and d (starting from the index finger) and one proximal triradius at the base of the hand, named the axial or *t* triradius. Sometimes (particularly in the abnormal hands of subjects with chromosomal abnormalities) there is another axial triradius placed more distally (nearer to the fingers) and named t' or t''. Having located the triradii, lines are drawn from each triradius, along the three ridges which meet at it, until these lines come to an end.

3. Dermal Ridge Dysplasia

Often in the hands of individuals with abnormal chromosomes the dermal (more strictly epidermal) ridges are ill-developed on fingertips and in the palm (particularly in the hypothenar area).

4. Skin Creases

A transverse palmar crease, running from one side of the hand to the other (the so-called "simian crease" because of its usual presence in monkeys and apes) is a common finding in individuals with chromosomal abnormalities and should be recorded (although it is present in some 10% of normal individuals as well). In addition, the number of flexion creases on the little finger is worth noting. Two creases is almost invariable in normal individuals, but a single one is often present in a variety of chromosomal aberrations.

B. Normal Dermatoglyphics

It is only possible to speak of normal and abnormal dermatoglyphics in a statistical sense. In any individual there will be

differences between the two hands; there will be differences between the hand of every normal (or abnormal) individual and every other; and there is considerable overlap between the normal and abnormal. So that while one may generalize and say that certain patterns are more common in certain abnormal conditions, this is not necessarily true in any particular individual and cannot be used as an absolute diagnostic feature.

The ridge-patterns on hand and fingers are laid down during the second month of intra-uterine life, and thereafter remain, for all practical purposes, unaltered.

It is pertinent at this point to consider what it is that causes the ridge patterns on the finger and hand to be as they are in any particular individual. Penrose (1968) has reviewed this problem and compares it to that imposed by laying a carpet covered with parallel lines on an uneven surface; or by combing a three-dimensional surface completely covered with hair; the mathematician Linwood observed that in order to comb the hair of a spherical dog completely flat it would be necessary to produce at least four whorls.

The mechanism which underlies the production of the ridge patterns is obscure, but their alignment suggests that it is related to compression forces operating at right angles to the lines, and the tension forces along their length; and that when the ridges are first formed on the embryonic finger, they tend to take the shortest course compatible with the direction of these forces.

Penrose (1968) has pursued the mathematics of ridge patterns further, and gives a formula which relates the numbers of triradii to the number of loops and the number of digits present; it may be used for checking a dermatoglyphic diagram to see whether all the relevant details have been included. The formula is as follows: On any limb, number of triradii + 1 = number of loops + number of digits (or, more strictly, number of nails; for if a nail is congenitally absent (anonychia) it is usually replaced by two loops, and many of the ridges completely encircle the finger-tip).

If "pattern-size" associated with an increased ridge-count is related to the size (or tension) of the fingertips as the ridges are formed, a low ridge-count will be indicative of retarded growth or of low tension in the fingertips at the time the ridges are formed, and conversely a high ridge-count will be indicative of high growth-rate or increased tension. Penrose (1967, 1968) suggests that an important factor may be the presence of fetal oedema at the relevant time. There is certainly supporting evidence for this in respect of Turner's syndrome. In this (see later) there is an exceptionally high

ridge-count, and it is also known that fetal oedema is usual (Singh and Carr, 1966), persisting to birth (Valentine, 1969) and gradually subsiding thereafter. But there is little evidence as to the presence or absence of fetal oedema in other chromosomal disorders.

However, it follows from all this that abnormal dermatoglyphics are indicative of a growth disorder at the time the dermal ridges are formed. Any condition leading to abnormal growth at this time will lead to abnormal dermatoglyphics, which are not therefore confined to or specific to chromosomal disorders; and conversely if growth is normal at this stage, the dermatoglyphics will be normal, whatever may happen later on (as for instance in a congenital condition such as phenylketonuria, which does not manifest its major phenotypic effects until after birth).

Finally, it must be remembered that there is great variation in ridge pattern in normal individuals, and this is strongly heritable; so that any abnormal dermatoglyphic patterns will always be superimposed on a very variable underlying pattern which has been inherited from the parents.

A diagram of a typical hand is shown in Fig. 57. The average percentage of the different types of pattern on the fingertips is given in Table 9 (after Taylor, 1969). It must always be borne in mind that the actual numbers in an individual may differ from this, but if a reasonable number of individuals are studied (say, 100) the aggregate figures will not differ widely from those given, as the author has been able to verify on the hands of Cambridge undergraduates. In the palm the axial triradius is typically near the base of the hand, and the

Table 9*

Fingertip pattern	Frequency %			
	Normal	Trisomy 21	Trisomy 18	Trisomy 13
Arch	5.0	2.7	87.0	38.4
Ulnar loop	63.5	82.8	8.0	40.1
Radial loop	5.4	1.8	4.0	11.1
Whorl	26.1	12.7	1.0	10.2
Palmar pattern				
Distal *t* triradius (atd > 57°)	8.5	82.9	62.4	90.4
Simian crease present	9.3	48.2	44.6	56.2

* After Taylor, 1969.

atd angle is acute (the norm is taken arbitrarily at 56° or less). The dermal ridges are normally developed, and there are two interdigital flexion creases on the little finger.

There is a strong hereditary likeness between the hands of members of families; this has been demonstrated by Holt (1961) for total ridge-count and (less strongly) by Penrose (1963) for *atd* angles. It is perhaps best shown in the comparison between mono- and dizygotic twins; the correlation coefficient for ridge-count in monozygotic twins is + 0.95, in contrast with + 0.49 for dizygotic twins. It is of interest also in monozygotic twins that the usual finding is that the right hand of one twin is more like the right hand of the other than the twin's own left hand (except in the less usual cases where there is mirror-imaging of the twins, where the reverse is true).

C. Dermatoglyphics in Chromosomal Aberrations

1. Autosomal Aberrations

(a) Down's syndrome. The dermatoglyphic features of Down's syndrome have been extensively studied (reviewed in Penrose and Smith, 1966). Cummins (1936) was the first to observe abnormalities in mongols.

(i) Fingertip patterns. Mongols characteristically show more ulnar loops present on average than in normal persons (Table 9)—around 80% of patterns are of this type.

(ii) Palmprints. There are unusual dermal ridge markings on the palm, particularly in the hypothenar region, and a distal axial triradius (t' or t'') is very common, so that the *atd* angle is increased (where two or more axial triradii are present, the angle is always measured at the most distal). Penrose (Penrose and Smith, 1966) has shown how the *atd* angle may be used to quantify the position of the t' triradius; but sex and age must be taken into account to do this.

(iii) Dermal ridge dysplasia. This is a common finding.

(iv) Skin creases. A simian crease is present in 48% (Table 9). A single interdigital flexion crease on the little finger is common (and no doubt related to the hypoplasia of the middle phalanx which is so common). The palm is often criss-crossed by many more creases than are found in normal people.

(v) Other features. A loop is more often found in the third interdigital area (between the bases of the middle and ring fingers) than in normal individuals (in 67% of mongols as against 36% of

normals). The dermatoglyphics of the sole of the foot are also abnormal. An arch (the tibial arch) on the ball of the big toe is the most characteristic feature (present on one foot in 50% of mongols, and less than 1% of normal Caucasians, although present in 6% of Orientals).

(vi) Dermatoglyphics in karyotypic variants of Down's syndrome. Penrose and Delhanty (1961) found a wider *atd* angle, on average, in balanced carriers of D/21 translocations, though Sergovitch *et al.* (1962) could not confirm this.

However, the most interesting findings in this field have been those on mosaic mongols. Penrose (Penrose and Smith, 1966) found that mosaic mongols were intermediate in respect of almost all dermatoglyphic features between full trisomic mongols and normal individuals. For example, ulnar loops on all fingers are found in 32% of mongols; in 23% of mosaic mongols; and in only 4% of normal persons. Penrose estimates that mosaic mongols showed a 61% deviation in their *atd* angle towards complete trisomic mongols.

Penrose also found that the mothers of mongols showed a 7% deviation towards mongols in their *atd* angles, and regarded this as consistent with 10% of the mothers of mongols being themselves mosaics; mothers of two mongols showed a 35% deviation, consistent with half of them being themselves mosaic. These figures are of particular interest since in most of these cases there is no cytogenetic means available by which a diagnosis of latent mosaicism can be made.

(b) Edwards' and Patau's syndromes. The findings in these two syndromes are summarized in Table 9. The peculiar feature of Edwards' syndrome is the very great number of arches found. Taylor (1969) states that fewer than three arches were never found in her series, and arches on all ten fingers were found in several cases. Whorls were very rare. A simian crease and increased *atd* angles were common. Dermal ridge dysplasia was found in 11.5% of digits. A single flexion crease on the little finger is very common.

In Patau's syndrome, two-thirds of cases have a simian crease; dermal ridge dysplasia is common (in 37% of digits). The distribution of fingertip patterns (about 40% each of arches and ulnar loops) is unlike that in any other condition. The *atd* angle was increased in 90%—a higher figure even than in Down's syndrome.

(c) Autosomal deletion syndromes. Information about dermatoglyphic abnormalities in these conditions is scanty, because of their rarity; but the following data are summarized from Taylor (1969).

(i) 4p– syndrome. Excess of arches, increased *atd* angle, simian crease, partial dermal ridge dysplasia (in 75%).

(ii) 5p– (cri du chat) syndrome. Increased *atd* angle in 2/3; simian crease in 50%.

(iii) Dq– syndrome (three cases). Simian crease in 2/3; distal *t* triradius in 3/3; excess of whorls.

(iv) 18p– syndrome. No dermatoglyphic anomalies.

(v) 18q– syndrome. Excess of whorls.

(vi) Gq– syndrome and anti-mongolism; no dermatoglyphic abnormalities.

(d) Summary of findings in autosomal anomalies. Findings of a distal palmar triradius and dysplastic dermal ridges seem to be fairly general, and are therefore probably non-specific effects produced by defective growth from an early age. However, the fingerprint patterns seem to have a certain specificity for different conditions which is not easy to explain.

2. *Sex Chromosome Aberrations*

Dermatoglyphic abnormalities seem to be less pronounced in sex chromosomal than in autosomal aberrations, but are nevertheless sometimes present. Apart from the special case of Turner's syndrome, where the stunting of growth (and fetal oedema) lead to a striking combination of large pattern-size with a high ridge-count on the fingertips associated with a rather transverse pattern of lines on the palm (with frequent simian crease and a *t* triradius which is abnormally distal, though less so, on average, than in Down's syndrome) the most striking feature is a negative correlation between the number of sex chromosomes present and the total ridge-count. This is shown in Table 10 (from Penrose, 1967). It will be noted that this only a tendency; the individual variability is very great, and the trend could only be detected by aggregating a large number of individual counts, because in any particular individual the trend is masked by the much greater inherited effect on pattern size. Nevertheless when the results are put in this form the finding is both unequivocal and striking. The explanation remains obscure, although, as already mentioned, Penrose has put forward the hypothesis that the ridge-count relates to the state of fluid balance in the developing embryo. There is evidence in plants and amphibia that chromosomal aneuploidy can give rise to alterations in fluid balance (Penrose, 1968); but apart from the fact that fetal oedema is known to occur in Turner's syndrome, there is little other direct evidence to support

Table 10*

Sex chromosomes present	Total ridge count Mean	S.D.	No. of individuals
X	178.6	44.0	40
XY	145.0	50.1	825
XYY	133.0	46.2	14
XX	127.2	52.5	825
XXY	114.8	49.6	25
XXYY	106.1	51.6	7
XXX	109.8	54.7	23
XXXY	93.0	49.2	9
XXXYY	73.0	–	1
XXXX	110.0	66.7	2
XXXXY	49.9	51.1	9
(XXXXX	0	–	1)

Note: The X chromosome seems to have at least twice the effect of the Y on pattern size reduction.

*After Penrose, 1967.

the hypothesis. Penrose has suggested that the frequency of cardiovascular anomalies in chromosomal aneuploidies may also be related to abnormal fluid balance, but again, apart from the finding of coarctation of the aorta in Turner's syndrome, it seems just as likely that the abnormal early growth found in most chromosomal aneuploidies is directly responsible for cardiovascular abnormalities, as that this is a secondary effect.

D. The Practical Use of Dermatoglyphics

It must be admitted that dermatoglyphics are not of great practical use for diagnostic purposes. Abnormal dermatoglyphic patterns due to disease are a secondary phenomenon, superimposed on a very variable primary pattern which is strongly heritable from the parents. Whenever the dermatoglyphics are so strikingly abnormal as to be almost diagnostic (as in Edwards' syndrome, where arches are so unusually common) it is likely that the diagnosis can be easily made clinically and confirmed directly by chromosome studies. So dermatoglyphics must be regarded as an interesting paraphenomenon, worthy of study in its own right, but of little practical clinical use. The only possible exception to this rule is where an individual is a latent mosaic and the abnormal cells cannot be detected by any cytogenetic means. Here it is possible that

abnormal dermatoglyphics could have a bearing on genetic counselling; but even so, they could not be given great weight. Also if a mosaic individual has abnormal dermatoglyphics, it implies that there was some disorder of growth at an early embryonic period; this being so, there should be other phenotypic abnormalities present, if these were systematically searched for (perhaps, for example, a lower stature than might be expected from the height of the parents) and these might be as valuable as the dermatoglyphic findings in diagnosis.

III. CHROMOSOME DISORDERS AND THE BRAIN

So little is known about the early embryology of the brain, particularly in quantitive terms, that it is impossible to give any account of what the actual developmental faults in particular chromosomal anomalies may be. However, it is possible to discern a few simple principles underlying the relationship between chromosomal disorders and faulty development of the brain.

The brain is the most sensitive indicator of any disturbance of development which occurs in embryonic and fetal life. This is because of the enormous complexity of the connections which have to be made by the various cells of the central nervous system during its early development. The processes of these cells have to travel long distances (sometimes several centimetres) in order to make connection with the cells on which they will later act; and these connections have to be made at the right moment in time, otherwise irreversible damage may be done to the whole system. For example, the damage done to the brain of a congenital cretin by the deficiency of thyroxin cannot be put right after birth by substitution therapy. A retardation of multiplication or growth of a particular group of cells in the central nervous system (CNS) may be enough to cause inevitable subsequent mental defect; and the causes of such retardation may be quite nonspecific; a virus infection, an overdose of irradiation or a chromosomal anomaly might all produce a very similar end-result if they affected a particular group of cells at a particular time. Thus, for example, the sort of brain anomalies found in cases of Patau's syndrome (arrhinencephaly, a tendency to fusion of the two sides of the forebrain adjacent to the central part) resemble very much anomalies which can be caused experimentally in fish and amphibian embryos by a number of treatments, such as hypoxia or alterations in tonicity of the environment. The only common feature is that all

such treatments have to be applied at a very early age—even during the first few cell divisions after conception—in order to produce these rather profound changes in forebrain development.

It seems evident, as a general rule, that the degree of mental retardation found in chromosomal anomalies is related to the amount of active chromosomal material which is either present in excess or absent. In autosomal disorders, the smallest amount of mental defect caused by a regularly-occurring condition is probably that found in Down's syndrome. In Down's syndrome the degree of mental defect varies greatly; the intelligence quotient (IQ) varies within a range from the 50s down to almost nil in different subjects. The most intelligent patients can just learn to read, and perform semi-skilled manual tasks. The great variation in IQ perhaps relates to the fact that every individual has a genetically different trisomic chromosome. The chromosome concerned (now called 21) is thought to be rather inert genetically; the degree of fluorescent staining (perhaps related to redundant genetic material) is high compared with the other chromosome of group G, which seems to support this concept. It may be that the most intelligent mongols have the least active genetic material on this chromosome; the least intelligent have more genetically active material, and thus suffer a greater degree of "genetic imbalance".

In other autosomal trisomies the degree of brain abnormality and of mental defect is much more profound; and in terms of morphological developmental abnormality the brain is more severely affected in Patau's syndrome (trisomy 13) than in Edwards' syndrome (trisomy 18). This again seems to relate to the size of the chromosomes concerned, and thus to the amount of redundant genetic material present in the trisomic condition. It may be noted here that as in trisomy 21, the trisomic chromosomes in Edwards' and Patau's syndromes seem to be the most fluorescent staining and the latest-labelling autoradiographically in their respective groups, indicating that they are perhaps the least genetically active chromosomes in those groups. Trisomy of the other, more genetically active chromosomes in those groups is not compatible even with survival to birth.

The sex chromosome anomalies shed further light on this point. Trisomy for the X chromosome, and the 47,XXY and XYY conditions may all be associated with mental defect; but not necessarily so. Greater numbers of sex chromosomes than this are usually associated with mental defect, but as reported elsewhere, one case of 48,XXXX has been reported with an IQ of 75. These findings

seem to lead to one or two further conclusions. First, they seem to indicate that the additional chromosomes do not exert their effect purely mechanically (a hypothesis which seems at first sight attractive). A 48,XXXX individual has far more additional chromosome material than any 47,G+ individual; but mongols never attain an IQ of even 75. Secondly, if the effect exerted by an extra chromosome is through gene action at some stage of development, the finding of mental defect in patients with X chromosome aneuploidy suggests that additional X chromosomes are not completely genetically inert, as the Lyon hypothesis originally proposed. The differences in the degree of mental impairment shown by different patients with X chromosome aneuploidies may relate to different degrees of genetic activity in a small segment of their heterochromatic X chromosomes.

Similarly differences in the mental (and physical) phenotype of patients with the 47,XYY syndrome may well relate to differences in the Y chromosomes. It would be surprising if the morphological differences which have been demonstrated between the Y chromosomes of different men (Chapter 2) were not sometimes reflected in a difference in active genetic content. So the spectrum of phenotypes shown by individuals with an additional Y chromosome may relate to the Y chromosomes concerned, the greater abnormality, both physical and mental, being in those individuals with more active genetic loci upon their Y chromosomes.

Perhaps what is most striking in mentally defective patients with chromosome anomalies is not how abnormal they appear, but how normal. Any individual who develops up until birth, and is capable of an independent existence, must be considered a very near miss at normality. Such development implies satisfactory development of brain connections of great complexity, relating to the lower centres in the brain which control the vegetative functions of the body. Such centres as the respiratory and vasomotor control centres are adequately developed and connected for normal or near-normal function. It is only the even more complex centres of the forebrain, which are responsible for the highest forms of mental activity, which are severely affected. These are always the regions of the brain most susceptible to any noxious influence.

Many gross anatomical and histological studies have been carried out on the brains of mongols (reviewed in Benda, 1946). Although many abnormalities have been found, none of these are very specific, and they do not shed much light on the developmental events which originally caused them, generally many years before. The only

obvious abnormality is the small size of the brain and the reduced total cell content (probably associated also with faulty neuronal connections). But these are rather non-specific changes, found in a wide range of conditions causing mental subnormality, and there is no one finding which can be considered specific to Down's syndrome.

Some rather more specific changes are found in the skull in Down's syndrome. Most skull changes in mentally subnormal patients are probably secondary to underlying abnormalities in the brain. The base of the skull may be considered as a capsule for the brain and for the sense organs such as the eyes and ears which represent extensions of the brain. Thus any abnormality of brain or of these sense organs is likely to be reflected in abnormalities of the skull which surrounds them and grows with them. However, in Down's syndrome there are probably some changes in the skull over and above this (Roche, 1966), which are due to the poor growth of the cartilage at the base of the skull (just as poor growth of cartilage in the rest of the body gives rise to the physical stunting which is such a marked feature of the condition). This hypoplasia of cartilage gives rise for example to the short, often upturned nose which is so characteristic, and through this to the epicanthic folds which are such a characteristic feature of the face.

To summarize what has been said: it seems evident that any excess or deficiency of genetic material in the cells of the brain will cause abnormal development, through some direct action of the excessive or deficient genes concerned; and that the severity of the consequent mental defect will be more or less proportional to the amount of active genetic material which is concerned in producing the "genetic imbalance". It follows that autosomal abnormalities will almost invariably cause mental subnormality; and that abnormalities of the sex chromosomes may well do so, depending on the amount of active genetic material which is present in excess (or deficient). This will depend on the individual X and Y chromosomes concerned, and may be minimal if they are almost entirely genetically inactive.

§13§

The Chromosomes and Acquired Disease

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I. CHROMOSOME BREAKAGE IN SOMATIC CELLS AND ITS SIGNIFICANCE

A. INTRODUCTION

In the literature of the last ten years many reports have appeared of chromosome breakage in cultures which come from individuals with certain diseases which may be genetic, infective or neoplastic; or who have been exposed to physical, chemical or biological mutagens; and also in cultures which have been exposed to such mutagens *in vitro*. It is not easy to evaluate the significance of these findings either in terms of the cells of the culture itself, or in terms of possible long-term effects on individuals exposed to these mutagens, and there has been considerable controversy on occasions (for instance as to the significance of chromosomal breakage following ingestion of LSD-25).

While it must be admitted straight away that little is known of the long-term effects on individuals, there are certain principles underlying the production of chromosome and chromatid breaks which need to be understood for the evaluation of such findings, and it is the purpose of this chapter to consider these. An excellent review of the subject has been written by Evans (1970).

The subject of chromosomes and neoplasia will be considered separately, for although it interlocks with the subject of chromosome breakage and repair it is as much concerned with chromosomal aneuploidy, and it has an extensive literature of its own.

B. Chromosome and Chromatid Breakage

The possible consequences of chromosome breakage as it affects the germ cells have already been considered (Chapter 8). This chapter is concerned with chromosome breakage occurring in somatic cells at some time during the life of the individual, and possible consequences thereof. Almost all studies on chromosome breakage have been carried out on cells in culture, and almost entirely on lymphocytes. As Evans (1970) has pointed out, lymphocytes are particularly good cells for such studies, since they are very long-lived, and do not normally divide while circulating in the bloodstream. Lymphocytes have been observed in cultures from persons who have been heavily irradiated by the atomic bombs dropped at Hiroshima and Nagasaki, which show chromosomal alterations of an unstable nature (such as the presence of acentric fragments) implying that the cells have been circulating, without having divided, for over 20 years. The mean lifespan of a small lymphocyte has been estimated as 500-1500 days, and these cells do not probably divide unless exposed to some substance, antigenic or otherwise, which "transforms" them and makes them divide, sometimes or perhaps usually with the consequent production of antibodies. (It is well known, for example, that in the phenomenon of rejection of tissue grafts from another individual the lymphocytes divide and produce an immune response which brings about the rejection phenomenon.)

1. Chromosomal Breakage and Rearrangement As Seen in Cultures

(a) Types of breakage seen. The sort of breakage which is seen after exposure to a mutagen, and any subsequent structural rearrangement of the chromosomes concerned, will depend on three things: the type of mutagen concerned; the stage in the mitotic cycle

at which the cell is exposed; and the time which has elapsed between exposure and chromosome preparation (relating especially to whether the cell has divided since exposure, and how many times).

The types of breakage which may be seen in a culture are sub-chromatid breakage, with or without loss of alignment, and whole chromosome breakage (Fig. 58). The breakage may occur at one or a few sites, or there may be multiple breakage up to the point of so-called "pulverization" of the chromosomes. If faulty reunion occurs, the various sorts of chromosomal aberration which have been described in Chapter 8 may be seen.

Fig. 58. Chromosome breakage (arrowed). (a) Chromatid breakage (or possibly an achromatic gap). (b) and (c) Whole chromosome breakage; (b) shows loss of alignment.

(b) Sub-chromatid breakage. This type of partial breakage of a chromatid has been shown to occur if cells are irradiated in early prophase of the cell division studied.

(c) Chromatid breakage. The appearance is shown in Fig. 58. If the fragments are still in alignment it may be impossible to distinguish between breakage and a simple achromatic gap (where continuity is preserved, but there is a non-staining segment of the chromatid). The two can only be distinguished with certainty at anaphase, when if the gap is a real one, the two parts of the

chromatid will separate. As Evans (1970) has pointed out, there has been little or no relevant work on anaphase chromosomes; however, perhaps the distinction is less important than it seems, since it has been found in work on plant chromosomes that achromatic regions of chromosomes are weak points at which breakage may subsequently occur.

If a cell with a chromatid break proceeds to a subsequent cell division the break will appear as a whole chromosome break (and has been called a "derived chromosome-type change").

Irradiation experiments show that chromatid breaks are found if cells are irradiated at the S or G2 phase. It is clear therefore that if they are found in a leucocyte culture, the actual breakage has occurred in the culture itself. If the break had occurred *in vivo* the division which took place in the culture prior to preparation would have converted it into a derived chromosome-type change.

(d) Whole chromosome breaks. The appearance is shown in Fig. 58. The break is seen at the same point in both the chromatids. Irradiation experiments show that in order to produce whole chromosome breaks, cells must be irradiated at the G1 phase, that is, before chromosome replication takes place. Thus if whole chromosome breaks are seen in culture they may indicate that the breakage has taken place *in vivo,* although they may also have taken place *in vitro* before the start of the S period.

(e) Chromosome pulverization. This term is used to describe the appearance of a cell in which the chromosomes have suffered multiple breakage, so that the chromosomes are only visible as a mass of fragments. Pulverization may follow cell death; or if caused by a mutagen, it will almost certainly lead to cell death.

(f) Chromosomal rearrangements following breakage. The subject of chromosomal rearrangement has already been considered, but needs brief discussion here in relation to how often chromosomal rearrangements are likely to be ascertained. The main types of whole chromosome aberration which may be found are listed in Table 11, together with the likelihood that they will be detected in chromosome preparations. Duplications, shifts and insertions, which represent three-break phenomena and are therefore probably uncommon, have been omitted. It can be seen from the table that the only aberrations which are likely to be ascertained with near 100% frequency are dicentrics and centric rings (and acentric rings in the first division after formation). At the other end of the spectrum, paracentric inversions will not be detectable at all by normal methods.

Table 11

Types of chromosomal rearrangement, and the likelihood that they will be detected (after Evans, 1970)

Rearrangement	Likelihood of detection
Deletion (terminal or interstitial)	Dependent on size
Centric ring + fragment	100%
Acentric ring + deletion	High in first division after formation; thereafter like deletion
Pericentric inversion	Depends if arm ratio is changed
Paracentric inversion	0%
Interchanges: symmetrical	0-20% (Evans, 1970)
Dicentric + fragment	100%
Centric fusion	100%

C. The effect of Cultural Conditions on Breakage

It will be clear from what has been said that it is most desirable that studies on chromosome breakage should be conducted on cells which are dividing for the first time *in vitro*. If cells are allowed to divide more than once *in vitro* chromatid aberrations will be converted into derived chromosome aberrations, and also the likelihood that any changes seen have occurred *in vitro* will be much increased. This means in practice that cells must be harvested after 48 hours instead of the more usual 72. Court Brown (1967) has found that many cells in 72-hour cultures are dividing for the second or even third time *in vitro*, whereas in 48-hour cultures all dividing cells are doing so for the first time in culture.

Any worker who has performed a fair number of leucocyte cultures will have observed that occasional cultures show quite large numbers of chromosome or chromatid breaks, or even pulverization. Repeat cultures on the same individuals do not usually show any abnormality, which indicates that the breakages are a consequence of cultural conditions; infection (e.g. by viruses or mycoplasma) has often been suspected, but seldom proved. Other possible factors might be variation or contamination of media, fluctuations in temperature and so on. It is therefore absolutely essential that any experiment on chromosome breakage should have the most rigid controls possible, particularly cultures set up from the same and other individuals at the same time as the experimental culture, and using identical media and conditions. It seems likely that much of

the disagreement which has arisen over the significance of various mutagenic agents has arisen because of the lack of adequate controls.

D. Spontaneous Frequency of Aberrations

Evans (1970) has estimated that in 48-hour cultures from normal people, and bearing the above difficulties in mind, the frequency of aberrations is as follows:

Chromatid-type aberrations:

Gaps 0.01-0.1 per cell
Deletions 0.0035-0.594 per cell (a wide range)
Interchanges < 0.001 per cell (perhaps 1 in 3000 cells)

Chromosome-type changes (much lower frequencies than chromatid aberrations):

Dicentrics about 0.00025 per cell (1/4000 cells)
Rings very rare

The frequency of aberrations is higher in 72-hour cultures. Sex and age probably do not have much influence (though there is disagreement on this point by Bochkov *et al.*, 1968).

II. MUTAGENIC AGENTS WHICH CAN CAUSE CHROMOSOME BREAKAGE IN VIVO *OR* IN VITRO

Mutagenic agents can be classified into three categories: physical, chemical and biological.

A. Physical Agents

The most important physical mutagen, and the only one about which there is a great deal of information, is ionizing radiation. There are other agents which can undoubtedly also be mutagenic, such as ultraviolet light and ultrasound, but information about their practical significance is rather sparse.

Much work has been done on the effect of ionizing radiation in producing chromosome breakage since the effect was first described by Muller (1927). The principles were worked out mainly on plant material in the 1930s and are well documented. They will not be further discussed here except inasmuch as they can be summarized by saying that the number of chromosome breaks produced (with possible consequential rearrangements) is broadly proportional to the amount of radiation received.

The effect of ionizing radiation on human chromosomes is of interest both in respect of persons who have received heavy doses of radiation, either for therapeutic purposes or accidentally (due to atom bomb explosions, and fallout) and in respect of individuals in the population who may have received relatively small doses, particularly during diagnostic radiology.

Studies on individuals who have received heavy doses of radiation have been carried out by Buckton *et al.* (1962) on patients who have received courses of radiotherapy for ankylosing spondylitis, and by Bender and Gooch (1962) on men accidentally exposed in the reactor disaster at Oak Ridge in 1958. Buckton *et al.* found that in their group the proportion of cells in culture showing unstable chromosome-type aberrations (dicentrics and tricentrics, rings and acentric fragments) was as high as 37% immediately after treatment and only fell very slowly with time; after one year 14% still showed such aberrations, and after six years there were still 4% (it will be remembered that the normal figure for such aberrations has been estimated at perhaps a fortieth of 1%). Stable aberrations (those transmissible without eventual cell death) were found in some 10% of cells, and this figure remained nearly constant over a six-year period. These figures indicate the very large number of chromosomes which are broken in the course of therapeutic radiation involving a fairly large body area; and confirm the long life of the lymphocyte in the peripheral blood (it was also noted that the total lymphocyte count was depressed after treatment, and remained relatively depressed for several years, gradually returning to normal).

However, quite small doses of irradiation given for diagnostic purposes can produce visible effects. This was first observed by Stewart and Sanderson (1961), who counted two dicentric chromosomes in 31 cells from a man with Klinefelter's syndrome who had had a diagnostic X-ray eight hours previously. Although the amount of irradiation received in the course of a chest X-ray is small, that received during fluoroscopy (in the course, for example, of a barium meal) is enough to produce a significant number of aberrations. Bloom and Tjio (1964) studied five patients 30 minutes after gastro-intestinal fluoroscopy and found four dicentrics in 300 cells.

In vitro experiments also show that quite small doses of radiation will produce significant numbers of aberrations. Evans (1970) estimated that 10 rads given to an *in vitro* culture would give one dicentric per 100 cells.

Ultraviolet light is, of course, absorbed by nucleic acids and will cause chromosome breakage. Since it cannot penetrate far into the

body, it will only, for practical purposes, affect cells in the superficial layers of the skin. Here it can certainly be mutagenic, as evidenced, for example, by the increased frequency of basal cell carcinoma (rodent ulcer) in persons much exposed to sunlight. But since the study of this in chromosomal terms would require skin cultures, the relationship between ultraviolet light and chromosome aberrations has not yet been quantified *in vivo*.

Ultrasound is another agent which has been claimed to produce chromosome breaks both *in vivo* and *in vitro*. Since ultrasound is becoming an important diagnostic tool in obstetrics and gynaecology, chromosome breakage could be of serious significance, particularly if it affected the unborn infant. Data both on *in vitro* and *in vivo* studies seem to be conflicting (Boyd *et al.*, 1971) and more experimental work is required before any possible effects can be evaluated.

B. Chemical Agents

Since Auerbach and Robson (1949) first demonstrated chemical mutagenicity, many substances have been demonstrated to be mutagenic either *in vitro, in vivo* or both. The distinction between *in vivo* and *in vitro* mutagenicity is an important one, since a substance which is known to affect chromosomes in culture may be detoxified in the body or fail in some other way to exert its effect; and conversely, a substance which is inert *in vitro* may be converted by the body into a mutagen.

A fair number of chemicals have now been conclusively demonstrated to be mutagenic *in vitro*; various groups of substances which may be important are cytostatic and alkylating agents which may be used in the treatment of tumours, or in immunosuppression (e.g. nitrogen mustard, myleran, piperazine, cyclophosphamide, imuran, etc.); antibiotics which are naturally-occurring (streptonigrin, actinomycin D, proflavine), various plant products (caffeine, theophylline, heliotrine, 8-hydroxyquinoline); DNA inhibitors or base analogues (cytosine arabinoside, deoxyadenosine, 5-bromodeoxyuridine, 6-mercaptopurine); and an assortment of other drugs and chemicals [benzene, plasma factor, lysergic acid diethylamide (LSD-25), cyclamates, mercury compounds]. References to work on these and other substances are in Evans (1970) and in books on chemical mutagenesis (Kihlman, 1966; Vogel and Röhrborn, 1970).

Only a minority of the substances which can produce chromosome breakage *in vitro* have been conclusively demonstrated to do so *in*

vivo. Among these, however, are a number of the cytostatic and alkylating agents, some DNA inhibitors, and methyl mercury (found in contaminated tuna fish). LSD-25 has been the subject of conflicting papers arguing for and against causation of *in vivo* chromosome breakage (McKusick, 1970). A method has now been devised for testing chemicals for *in vivo* mutagenicity by means of a host-mediated assay after toxification or detoxification by the mammal concerned (Vogel and Röhrborn, 1970) and by this method some substances have been cleared of *in vivo* mutagenicity (for example, caffeine—to the general relief of cytogeneticists) and others have been shown to carry a potential risk (DDT, cyclohexylamine). The subject is still in its infancy, but it is clear that much will be heard of it in the next few years, and that many recently-developed and powerful chemical agents will need screening by such techniques.

Chemical mutagens generally resemble biological mutagens and differ from physical agents in that the effects tend to be localized to particular chromosomes and chromosomal regions (such as constrictions and heterochromatic areas), while with ionizing radiations breakage tends to be randomly distributed.

C. Biological Agents

Nichols *et al.* (1962) were the first to demonstrate chromosomal abnormalities in cultures from patients with virus diseases. They found chromosomal breakage in cultures from patients with measles. Since that time it has been shown that a number of viruses can cause chromosomal breakage in cultures, either when added to the culture or when cultures are made from patients with virus diseases; viruses thus incriminated include measles, chickenpox (Aula, 1963, 1965), yellow fever vaccine (Harnden, 1964), herpes zoster and adenoviruses, polyoma virus and a number of viruses associated with neoplasia such as the Rous sarcoma virus, Shope papilloma virus and others. The subject has been reviewed by Nichols (1969).

The types of aberration produced in cultures are chromatid and chromosome gaps and breaks (possibly different stages of the same process), pulverization, and sometimes cell fusion associated with anomalies of the spindle. Pulverization is an appearance in which there is a mass of small chromosomal fragments, sometimes scarcely or not recognizable as chromosomal. Nichols *et al.* (1967) have shown that the fragmentation takes place during the S-period. The appearance is sometimes seen in supposedly normal cultures, probably as a consequence of cell death. Cell fusion can occur with

some viruses, and Harris (1965) has utilized this phenomenon, induced by inactivated Sendai virus, to produce fusion of cells from different species; the technique has proved a potent one in the investigation of the relationship of chromosomes to malignancy.

The mechanism of chromosome breakage by viruses is not known with certainty. The resemblance of the effects to those produced by chemical mutagens, particularly DNA inhibitors (especially in the sites of breakage), has suggested that breakage may be related to inhibition of DNA synthesis; but this is not proven. Other theories put forward are reversal of the DNA polymerase reaction, breakdown of lysosomes [though Aula and Nichols (1968) consider this unlikely as there is no increase in acid phosphatase in the cells] and arginine depletion. Arginine depletion is produced by mycoplasmas (pleuropneumonia-like organisms, PPLO) which are known to be capable of producing chromosome breaks in culture, which can be prevented by added arginine. But in sum it is not known what the main mechanism of chromosome breakage by viruses may be; more than one of the mechanisms just quoted may be involved. In conclusion it may be noted that non-infectious virus fractions can induce pulverization, so active multiplication of the virus is not necessary to produce effects.

As with chemical agents, so with biological ones, it is not easy to assess the significance of these findings *in vivo*. As Evans (1970) has pointed out, even if cultures from patients with virus diseases do show chromosomal breakage, it is necessary to show that the cells concerned have passed through the S or G2 stages *in vivo*. This can only be shown with conviction on cells obtained from bone-marrow, and there is a dearth of studies on these. Thus although there can be no doubt that biological agents can affect chromosomes *in vitro* the practical significance of this remains obscure. Nevertheless it must be admitted that viruses must be regarded as potential *in vivo* mutagens, and any virus which has caused chromosomal aberrations in culture (which includes measles, chickenpox, herpes simplex and zoster, aseptic meningitis, mumps, infectious hepatitis and yellow fever) must be regarded with suspicion.

III. CLINICAL CONDITIONS ASSOCIATED WITH CHROMOSOME BREAKAGE

Apart from the overt infections which have been mentioned as causing chromosomal breakage, there are a number of other clinical conditions in which associated chromosomal aberrations have been

reported. These unrelated disorders, all of which appear to be inherited as recessive traits, and which are associated with chromosomal aberrations, are Fanconi's anaemia, Bloom's syndrome and ataxia-telangiectasia.

Fanconi's anaemia (perhaps a better name is pancytopenia) is a condition where there is a general depression of bone-marrow function; there are also various congenital anomalies. G. E. Bloom *et al.* (1966) have demonstrated chromosome breaks in cultures from patients. These are not so readily demonstrated in marrow cells (Schmid, 1967b). Bloom's syndrome (D. Bloom, 1966) consists of dwarfism with sensitivity of the skin to sunlight, and again multiple chromosome breaks are found in cultures (German *et al.*, 1965). Ataxia-telangiectasia is a condition characterized by multiple telangiectases, progressive cerebellar ataxia and a deficiency in the immune mechanism with hypoplasia of the thymus. Again, chromosome breaks are found with high frequency in cultured fibroblasts and leucocytes. It is perhaps significant that in two out of the three conditions there is certainly immunological deficiency. Another very significant common feature of all three conditions is that the subjects are prone to malignant disease, particularly leukaemia.

Two possible explanations of the high frequency of chromosomal breakage in culture immediately spring to mind; the first is that the breakage may be due to a biological agent (viral or otherwise) which can flourish in culture (and possibly *in vivo*) because of the impaired immunological mechanisms. The other is that there is some primary deficiency in the leucocytes themselves, due to the abnormal gene present, which is responsible for both the chromosomal breaks and the deficiency in immunity. Whether *in vivo* breaks occur is not certain, but the frequency of malignant change in all three conditions is perhaps circumstantial evidence for chromosomal aberrations in the bodies of the sufferers. Another explanation which has been suggested for the chromosomal breakage is abnormal lysosome activity (Swift and Hirshhorn, 1966).

Chromosomal aberrations have also been reported in patients with vitamin B_{12} or folic acid deficient megaloblastic anaemia. Here aberrations have been demonstrated in direct preparations of uncultured marrow cells, indicating a definite *in vivo* effect. As well as increased chromosome breakage, spreading and breakage at the centromere and incomplete contraction and elongation at secondary constrictions have been reported, as well as increased numbers of hypodiploid cells (Kiossoglou *et al.*, 1965; Heath, 1966; Menzies *et al.*, 1966). Menzies *et al.* consider that the abnormalities are probably

due to abnormal DNA metabolism, since it is possible to demonstrate a block of DNA synthesis in marrow cells in this type of anaemia, consequent on vitamin B_{12} and folic acid deficiency; with replacement of these vitamins all the clinical and chromosomal abnormalities disappear.

IV. CHROMOSOMAL ABERRATIONS AND NEOPLASIA

A. Introduction

The relationship of chromosomal aberrations to the formation and development of new growths of any kind, whether solid tumours or neoplastic processes involving haemopoietic or other free-living cells, is a complex one, and it is only now, after many years of patient research, that patterns of relationship are beginning to be observed. The basic difficulty in studying the subject is that every new growth is different and that although chromosomal aberrations are seen in tumours with great constancy, it is difficult to relate them to the type of tumour or the way in which it is behaving. There is only one notable exception to this general rule, and that is the rather constant finding of a specific chromosomal aberration, the deletion of a chromosome of the G group, in the stem cells of chronic myeloid leukaemia. This is called the Philadelphia or Ph_1 chromosome. Apart from this one instance it is not possible to say that any particular form of new growth is associated with a specific chromosomal anomaly.

However, knowledge of the subject has been built up over the last 15 years partly by observations on the chromosomal composition of human growths, particularly in the leukaemias and related disorders, but also in solid tumours, and partly by patient observation of, and experimentation with, various animal tumours which have been studied for long periods either by transmission from one experimental animal to another, or by growth *in vitro*. The findings are complex, and would require a complete volume to do them justice; so the following account must only be regarded as a very brief outline.

B. Chromosomes of Experimental Tumours

The subject has recently been reviewed by Levan (1969), who is a major pioneer authority on the subject. As a start, the generalization may be made that while normal tissues almost invariably have normal

chromosome complements (with the proviso that there is a tendency to increasing aberration in ageing tissues), virtually all neoplastic tissue shows deviations from the normal if these are carefully searched for. And in general, the longer the abnormal tissue has been growing, the greater the number of abnormalities of chromosomes that are found. Even if perfectly normal cells are grown in culture for long periods, they tend to alter their character and to show an increasing tendency to disturbance in their chromosome complement.

It may be taken as axiomatic that the chromosomal changes which take place either in normal cells in long term cultures, or in tumours as they grow and progress, are related in some way to cellular adaptation to an abnormal environment. The process may be regarded as rather similar to natural selection in populations of animals living in changing environment. Mutations take place in certain cells; under normal circumstances in the body most of these are probably immediately suppressed, either by immune or other mechanisms; but in the uncontrolled environment of culture or tumour these mutated cells can compete on equal terms with their neighbours. Certain of them may have genetic advantages, and form clones of cells descended from them, which increase in number relative to the cells surrounding them. If this process is repeated, the eventual picture is of a mass of cells comprising a number of different lines which may be distinguished by their chromosomal composition. Often there is a major stem-line which predominates, and a number of subsidiary stemlines. The older the colony of cells, the more complex the situation may become. From the point of view of the experimentalist the problem is to relate the chromosomal changes which are seen to the adaptive changes which occur in cultures of tumour cells.

In experimental mouse tumours there is a tendency for the chromosome complement to shift towards a tetraploid, or slightly sub-tetraploid chromosome complement, and this seems to be associated with increased vigour and capacity to withstand environmental changes. Perhaps the increased chromosome complement gives an increased capacity for genetic change or chromosomal evolution in a varying environment. Unfortunately the uniform telocentricity of the normal mouse chromosomes makes it difficult to determine whether particular chromosomes are more favoured than others in specific situations.

Kato (1968) studied 42 Chinese hamster primary tumours. This animal is good for cytogenetic experimentation because it has a low

diploid chromosome complement (22) and wide morphological variation between individual pairs. In the tumours, besides the 42 stemline karyotypes there were 26 sidelines. The total number of different karyotypes found was 28, of which 22 were basically diploid and six tetraploid. The commonest aberration in the diploid series was the addition of one morphologically normal chromosome to give a trisomic karyotype and in these trisomic lines, only three of ten possible autosomes were involved; a very clear instance that the aberrant karyotypes were not produced at random, but that particular chromosomes were selectively favoured. In tetraploid lines there was much more structural variation, with the appearance of marker chromosomes.

Levan similarly found in rat primary tumours that about half showed a trisomic stemline which included an extra telocentric chromosome. The general pattern of behaviour in these and the Chinese hamster tumours was of a normal diploid stemline becoming accompanied by a trisomic stemline, which gradually increased in size until it took over from the diploid stemline as the principal stemline of the tumour.

Recent experiments by Harris on the hybridization of tumour cells by use of the Sendai virus (see p. 288) also indicate that the chromosome complements of tumour cells are not random, and the hybridization technique (especially when used to hybridize the cells of different species) promises to be a potent one for this line of research.

It must be admitted, however, that although these karyotypic changes are usually associated with tumour formation and development, it cannot be said whether they are in any way causative of tumour formation; they may be a secondary phenomenon which follows the primary change which initiates the tumour. However, they clearly play an important part in the subsequent evolution and behaviour of the tumour in which they occur.

C. Chromosomes and Human Neoplasia

1. Introduction; Karyotypes of Solid Tumours

Human new growths may be divided into those which form solid tumours (carcinomas, sarcomas, etc.) and those in which the neoplastic cells are more or less free-living and widely disseminated throughout the body. From a research standpoint, solid tumours do not make good experimental material, for several reasons. First there is the difficulty that because they often grow relatively slowly, and

contain much degenerating tissue, it is not always easy to find adequate numbers of dividing cells to study; and secondly, and more importantly, it is seldom if ever possible to make serial observations on their karyotypic composition, since it is necessary for the patient's sake to remove any large masses of tumour which can be removed, as soon as possible (or to treat them by irradiation where this is not possible); and even if this is not possible a series of biopsies for experimental purposes would be ethically unjustifiable. Despite this, many reports have been published on tumour karyotypes. In general, a normal karyotype has not been found; single stemlines are the commonest finding, and these are usually near diploid, though variants ranging from hypodiploid to tetraploid have been described. Structural chromosomal changes are common. In general it is not possible to relate a specific chromosomal abnormality to a specific type of tumour; however there are suggestions that this may occasionally be possible; for example, a characteristic marker chromosome has been found in several primary Burkitt tumours (Jacobs *et al.*, 1963; Stewart *et al.*, 1965) and eight human meningeiomata all had hypodiploid stemlines with one missing G chromosome (Zang and Singer, 1967). While further examples of this sort are likely to be found, it does not seem profitable to pursue the problem of solid tumour karyotype further here, when so much more information is available in respect of leukaemias.

2. Chromosomes and Leukaemia

By contrast with solid tumours, the leukaemias afford very favourable material for cytogenetic study. Direct preparations may be made from the bone-marrow, thus obviating possible artifacts due to cultural conditions, and repeated specimens may be available in which the progress of the karyotype of the abnormal cells in relation to the disease can be followed (since the clinical assessment of the condition in relation to possible treatments entails continuing study of bone-marrow cells).

(a) Chronic leukaemias. The most dramatic cytogenetic finding in any form of leukaemia is that already mentioned, of the usual presence of a deletion of the long arm of a G group chromosome in the stem cells in chronic myeloid leukaemia, first reported by Nowell and Hungerford (1960) working in Philadelphia, and now by common agreement referred to as the Philadelphia or Ph_1 chromosome. It was long thought that the chromosome concerned was No. 21 (Denver), the chromosome trisomic in Down's syndrome, for two main reasons; first that leucocyte alkaline phosphatase activity is

raised in Down's syndrome and lowered in chronic myeloid leukaemia, suggesting involvement of the same chromosome; and secondly the well-known predisposition of mongols to develop leukaemia which again focused attention on a common factor, chromosome 21, although not suggesting an explanation. However, O'Riordan *et al.* (1971) have recently produced evidence from fluorescent staining that the deleted chromosome is actually No. 22.

The Ph_1 chromosome is not always to be found in cultured leucocytes from cases of chronic myeloid leukaemia, particularly after treatment, probably because normal cells are more likely to be transformed in culture than leukaemic cells; but it can usually be found in the marrow even during remissions, reappearing in the blood when the conditions worsen; during the so-called "blastic crises" which occur, in which various white cell precursors appear in the blood stream, cell lines with additional chromosomal abnormalities may appear (Court Brown and Tough, 1963; Hammouda *et al.*, 1964). Furthermore there are some cases of chronic myeloid leukaemia in which the Ph_1 chromosome is never found. In childhood, under the age of 15 years, two forms of the disease can be distinguished, a juvenile form and an adult form. Hardisty *et al.* (1964) consider that these two forms can best be distinguished by the presence or absence of the Ph_1 chromosome; it is absent in the juvenile form, which is characterized by a lower leucocyte count than the adult form, thrombocytopenia, and an increased level of haemoglobin F (15-55%). Ph_1 negative adult cases have also been reported (Tjio *et al.*, 1966) and these also appear to be characterized by a relatively low white cell count, poor response to treatment, and short survival.

In the other major type of chronic leukaemia, chronic lymphatic leukaemia, chromosomal aberrations seem to be the exception rather than the rule. An early report by Gunz *et al.* (1962) of an association with an abnormal chromosome (Gp−) in a family with chronic lymphatic leukaemia has not been generally confirmed. It may be that the common finding of a normal karyotype in patients with this form of leukaemia is due to the fact that only normal cells are transformed in culture. In another neoplastic blood disorder, Waldenstrom's macroglobulinaemia, an extra autosome has been reported in a number of patients (Bottura *et al.*, 1961; Elves and Israels, 1963). It resembles a No. 2 chromosome morphologically. Chromosomal anomalies have also sometimes been noted in Hodgkin's disease, but no constant pattern has been observed.

(b) Acute leukaemia. The karyotype in acute leukaemia is found

to be abnormal in some 50% of cases, and the abnormalities appear to follow a pattern which is somewhat reminiscent of the findings in experimental tumours which have been outlined above. Sandberg *et al.* (1964) studied the chromosomes in 75 cases of acute leukaemia. In 41 cases of acute lymphatic (or lymphoblastic) leukaemia (39 of whom were aged under 14) they found aberrant chromosomal lines in 21, and all of these were hyperdiploid, with a count between 47 and 51. In 34 cases of acute myeloblastic leukaemia, however (of whom five were under the age of 14), they found aberrant lines in 14, and the majority of these were hypodiploid with chromosome counts ranging between 41 and 48. The chromosomes most commonly involved in the aneuploid lines were members of groups C and G. These findings certainly appear significant and other workers have since found rather comparable aberrations (Walker, 1970).

(c) Conditions predisposing to leukaemia. There is now a considerable body of evidence to the effect that circumstances predisposing to chromosomal breakage lead to an increased incidence of leukaemia. The examples of Fanconi's pancytopenia, Bloom's anaemia, and ataxia-telangiectasia, all conditions in which chromosome breaks are found with greater frequency than normal in cultures, and in which there is an increased liability to develop leukaemia, have already been mentioned. Furthermore it is now well documented that exposure to ionizing radiations is a predisposing factor. This was shown by Brill *et al.* (1962) in respect of survivors of the atomic bomb explosion at Hiroshima, and by Court Brown and Doll (1965) in patients irradiated for ankylosing spondylitis; and it has even been shown that irradiation of pregnant women carries a risk of leukaemia for the unborn infant. Benzene can cause chromosomal aberrations, and can be leukaemogenic (Tough and Court Brown, 1965; Vigliani and Saita, 1964). Two interesting reports have appeared describing individuals who were exposed to whole-body irradiation, and who showed abnormal deleted G group chromosomes indistinguishable from the Ph_1 chromosome, but who had not developed leukaemia at the time this was found. Goh (1966) reported such chromosomes in three patients who had been treated with whole body irradiation for ankylosing spondylitis, and Kamada *et al.* (1970) reported the same phenomenon in heavily-irradiated atomic bomb survivors. It will be very interesting to know if any of these individuals subsequently develop chronic myeloid leukaemia. It may well be that work on other physical and chemical mutagens will also prove that they engender increased liability to leukaemia, but the relevant statistics are not easy to come by. Finally, the well-known association

between Down's syndrome and leukaemia must be mentioned once again in this context.

It is difficult to avoid a conclusion from all this evidence that leukaemia may result as a direct consequence of chromosome breakage, but there is no firm evidence as to what the triggering event may be.

Finally, it should be mentioned that there appears to be a genetic factor predisposing some individuals to develop leukaemia. MacMahon and Levy (1964) have shown that an identical twin has an increased tendency (1/9) to develop leukaemia within a few months of his or her "other half" doing so. And there are a number of instances where the incidence of leukaemia in a family is much higher than might be expected by chance.

(d) Chromosomes and the cytology of neoplastic cells. Neoplastic cells may show considerable variation from the normal as evidenced by both light and electron microscopy (Bernhard, 1969). Some of these changes are relevant to the role of the chromosomes in malignancy. Nuclear structure is affected in neoplasia; the nucleus is often enlarged (presumably in relation to an increased chromosome complement) and the outline is irregular; the nuclear membrane shows deep infoldings or bleb formation, and cytoplasmic inclusions may be found within the nucleus. Conversely, parts of the nucleus may be cut off from the rest, to form separate masses of chromatin surrounded by their own nuclear membrane; this could be one of the mechanisms by which the chromosome complement is altered following cell division. There is not, however, any visible difference in the structure of the chromosomes themselves as seen under the electron microscope.

There are also cytoplasmic abnormalities seen with the electron microscope. The mitochondria are often structurally abnormal. Endoplasmic reticulum and Golgi apparatus are usually poorly developed, but the number of free ribosomes is increased, as it is in many cells of the developing embryo.

The changes in the nuclear membrane are of particular interest in view of the part chromosomes appear to play in organizing its formation, and the relationship which the ends of chromosomes probably have to it (Chapter 6). It is possible to speculate that the role of the Philadelphia chromosome in chronic myeloid leukaemia may be related to the function of the deleted end *vis-a-vis* the nuclear membrane. The chromosome concerned is such a small one that the deletion does not cause cell death (as most chromosomal deletions of any size may be expected to do); but it may cause a fatal

interference with the proper formation and functioning of the nuclear membrane.

D. Summary

The subject of chromosomes and neoplasia may be summarized by stating that new growths almost always have abnormal chromosome complements, except perhaps at the very beginning of their life; that these abnormalities evolve during the life of the tumour, so that new stemlines emerge from time to time; that the chromosomal patterns cannot usually be directly related to the nature of the growth (with the notable exception of the Ph_1 chromosome found in chronic myeloid leukaemia), but that there are strong suggestions that some patterns are commoner than others, and that the patterns do have a definite functional significance for the growth; and finally, that it is not yet possible to say whether the chromosomal changes are the cause or the result of the neoplasia, although the known tendency to leukaemia in some conditions where more chromosome breakage than normal is found suggests that in this form of neoplasia chromosomal changes could be a causal aetiological factor.

§ 14 §

Chromosomes, Medicine and the Future

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I. INTRODUCTION

Cytogenics is merely the handmaiden of its parent science, genetics, and although it can be treated, as it is in this book, as a subject in its own right, its usefulness in the long run depends on what it can contribute to our whole knowledge of the science of heredity. The eventual ideal range of knowledge which all geneticists must aspire to obtain consists, at least, in the ability to analyse the complete nucleotide sequence which characterizes a particular individual; to know in which chromosome each item of genetic information resides; to know when each bit of genetic material is active, and how it works, during the life of the individual from conception to death; and to know how the genetic material segregates during the formation of the gametes, which are to pass it on to future generations. This last will only be expressable in terms of probability. Such an idea may sound impossible at present, but if the human race survives for an indefinite period and continues to make scientific progress at the rate at which it is doing at present, it is not at all impossible that something very close to it may be achieved.

Cytogenetics can only play a relatively small part in the realization of such an ideal. What it can offer is the possibility of individual identification of each human chromosome; and geographical location of genetic material on the chromosomes, giving the possibility of

visual study of particular loci and of their activity, and also of the way in which these loci segregate at meiosis.

If so-called "genetic engineering" (that is, the substitution of new genes for those already there, or the insertion or removal of particular genes) ever becomes possible in higher organisms on anything but a very minor scale, it is certain that detailed cytogenetic knowledge will be an indispensable prerequisite. Such a possibility certainly seems remote at present, because of the complexity of the eukaryote cell; but knowledge is accumulating so rapidly at present that the possibility of some form of genetic engineering in the foreseeable future cannot be discounted.

II. CHROMOSOME MAPPING

The mapping of the positions of genetic loci on chromosomes has been extensively carried out in *Drosophila.* The advantages which this species affords in terms of rapid breeding, large number of known genetic loci with distinctive expression, and the possession of polytene chromosomes with distinct banding patterns which can be used for the geographical location of the genetic loci, have been thoroughly exploited. The oldest and most widely used method of chromosome mapping depends on the demonstration of genetic linkage between two loci; that is, that the two loci are situated on the same chromosome, and are reasonably close to each other. If this is so, then the actual distance can be estimated by discovering the proportion of occasions on which a crossing-over occurs between them during meiosis. This is expressed in terms of map units (centimorgans, recombination units). One unit is the linear distance on a chromosome within which there will occur on average one cross-over per 100 gametes.

Chromosome mapping also involves locating linked genes on a particular chromosome. This is not too difficult in *Drosophila,* because of the small number of chromosomes and large number of genetic loci involved; but it is extremely difficult in man, where the reverse is true. Until very recently the only genes in man which could be assigned to a specific chromosome have been those located on the X chromosome. Location of genes on the X chromosome is possible because of the special nature of X-linked inheritance, which enables geneticists to say with reasonable certainty that genes which are inherited in a particular manner must be located on the X chromosome. Seventy or more of such genetic loci have been

identified (McKusick, 1965). In Chapter 15 there is given a recent map of the X chromosome.

The first autosomal linkage to be discovered in man was the ABO blood group–nail-patella syndrome linkage discovered by Renwick and Lawler in 1955; and the first location of any genetic locus to a particular autosome was the assignment of the Duffy blood group locus to chromosome No. 1 by Donahue *et al.* (1968). Since that date new techniques have made possible several more such assignments; the present state of knowledge is summarized by Renwick in the last chapter of this book.

The basic principle underlying the measurement of linkage is quite straightforward. If two genetic loci are close to each other on the same chromosome they will usually segregate and be inherited together; but occasionally a crossing-over occurs between them and only one is inherited. Thus, for example, if women can be found who have two genes known to be present on the X chromosome, and who are know to have both of these on the *same* X chromosome (as shown by having inherited them from a parent who possessed both) then, if the genes are closely linked, they will usually be transmitted together (or not at all) to the offspring. Occasionally, however, a child will be born with only one of the two genes. The frequency with which this event occurs will give an indication of the closeness of the linkage. Of course, individual sibships will not give enough information, but if a number of sibships of this sort can be studied, in many families, the data can be aggregated and an estimate of the distance between the two loci can be made.

In practice things are not so easy. If the two loci are far apart, crossing-over between them may occur more than once, and although this possibility can be corrected for mathematically, it lowers the reliability of estimation. In general, distances above 25 centimorgans cannot be estimated with reasonable accuracy (distances above 50 centimorgans cannot, of course, be measured at all). Since recent estimates of the genetic length of the X chromosome put it at as much as 250 centimorgans, it may be some time before linkage can be established between many of the loci known to be present even on this chromosome, particularly as many of these loci are rare, so that the likelihood of finding informative families is low. Luckily there is one really common X-linked gene, that for haemoglobin Xg. The gene frequency for the locus Xg(a+) is about 0.66 (66%), and so it has proved as valuable in linkage studies as it has for studies of aneuploidies involving the X chromosome. Unfortunately it seems likely that the locus is situated near one end of the X chromosome, so that fairly few of the known X-linked genes are close to it.

The mathematics of working out linkage from pedigree data is complex and lies outside the scope of this book. The method commonly used is called the lod score method and its use has been reviewed by Maynard-Smith *et al.* (1961) and Renwick (1969). The method will give the probability that two loci are linked, and give an estimate of the distance between them (in centimorgans) if they are linked. It will not, of course, give any indication as to the relative position of two linked loci, and as to which side of each other they lie in relation to other loci which may be linked to either of them; these facts can only be deduced from patient analysis of all estimates of distance between all pairs of linked loci within range.

The information which such techniques have given us is summarized by Dr J. H. Renwick in Chapter 15. New and powerful techniques have recently been evolved for locating genes on particular chromosomes; in particular the technique of fusing cells from different species by use of the Sendai virus (Harris, 1965). If a cell from a line deficient in a particular enzyme is fused with a cell of another species, all the chromosomes of the second cell may be eliminated except for that which contains the locus for the enzyme in question.

The knowledge of genes on chromosomes summarized in Chapter 15 has taken a vast number of man-hours to accumulate; work on the map of the X chromosome has been proceeding for more than a generation. But the introduction of such techniques as those described above, and also the introduction of the fluorescent and pretreatment-Giemsa staining techniques, which offer the possibility of individual identification of the human chromosomes, is likely to cause a rapid acceleration of our knowledge of the human chromosome map.

III. THE CONTRIBUTIONS AND THE COST OF HUMAN CYTOGENETICS

A. Introduction

The contribution made by the advances in human cytogenetics over the last 15 years can be considered in two separate categories. First is the contribution to scientific knowledge in general and to the science of genetics in particular; and secondly, the contribution to human welfare, in terms of happiness and of economic advancement.

There is no doubt as to the value of the contribution which our knowledge of the human chromosomes has made to the science of

human genetics. It has clarified many problems which were previously unsolved, and has led, in conjunction with other recently-developing subjects such as molecular biology, to a much deeper understanding of the detailed mechanism of heredity, and into ways in which this can go wrong. However, when the positive contribution which cytogenetics has made to human welfare is analysed, it appears very much less impressive, and could certainly be criticized by economists on grounds of cost-effectiveness.

B. The Contributions of Human Cytogenetics to Medicine

The practical contribution of cytogenetics to medicine may be summarized by saying that it has enabled a more accurate diagnosis to be made of a number of conditions; and although it offers at present no prospect of curative treatment, it has enabled physicians to make more accurate prognoses of affected individuals from the time of birth onwards (or even before birth) and to offer better advice. In addition to this, there are a number of ways in which prospective parents can be helped by the giving of advice upon which action can be taken; these fall under the general heading of genetic counselling, the scope of which has recently been enlarged by the possibility of selective abortion of unwanted children. In addition to all this, the knowledge which has been gained of acquired chromosomal changes enable these to be used as an index of mutagenesis for various mutagenic agents, and to a limited extent in the prognosis of some forms of neoplastic disease.

These various uses of cytogenetic knowledge will now be considered in greater detail.

1. The Value of Cytogenetics in Diagnosis and Prognosis

On the whole, cytogenetic diagnosis adds little to help in the management of individuals with autosomal disorders. The commonest of these, Down's syndrome, is easy to diagnose without cytogenetic analysis, and a knowledge of whether the underlying abnormality is a straightforward trisomy, an unbalanced translocation, or a mosaic condition, does not really add anything to prognosis or treatment (although it may be of value when giving genetic counselling to the parents). The same considerations apply to most other autosomal abnormalities.

There is, on the other hand, some value in the cytogenetic diagnosis of abnormalities of the sex chromosomes. For a start, some of these may not be readily diagnosable without cytogenetic analysis

(this will include some cases of 47,XXY and probably many of 47,XXX and 47,XYY). This suggests that cytogenetic screening at birth might be of value in the detection of such cases, so that the parents could be given appropriate advice. Now that the detection of the fluorescent Y-body in interphase nuclei is possible in addition to the detection of the sex chromatin body, it should be possible to detect the majority of abnormalities of the sex chromosomes at birth without the expense and difficulty of a full chromosome analysis.

It must be admitted, however, that widespread screening of newborn infants to detect sex chromosome abnormalities might create more problems than it solved. What action should be taken if a sex chromosome abnormality is found?

The simple answer to this question is probably that the parents should be told of the diagnosis, and of the likely prognosis. But there are snags in this course of action. First, the accuracy of the diagnosis must be as absolute as possible. It would be a disaster to suggest that a normal child might have a chromosomal disorder. But this is quite possible in a massive neonatal screening programme. Such accidents are almost certain to occur periodically, on purely statistical grounds. Doctors are only too painfully aware, from reading the annual brochures from their defence organizations, of the accidents which occur with monotonous regularity in a large organization such as the National Health Service; the wrong digits which are amputated; the swabs left in the abdomen after operations; even the mother who leaves a maternity home with the wrong baby. So it would be essential to ensure that there were stringent checks on any diagnosis of a chromosomal abnormality which might be made on a newborn child. There is a particular pitfall in the diagnosis of chromosomal disorders, and that is the possibility of undetected mosaicism. Even in the presence of a sex chromosome abnormality, the presence of an undetected normal cell line might lead to a much better prognosis than expected.

Even if the cytogenetic diagnosis was absolutely firm, it would be difficult to know what advice to give, and this applies particularly to three out of the four commonest sex chromosome aneuploidies.

(a) 47,XXY. While it is almost certain that the sufferer from Klinefelter's syndrome will be sterile, there are recorded cases of 47,XXY individuals with no other detectable cell line who have children. In view of the fact that one in ten marriages between apparently normal persons are sterile, is there any advantage in telling the parents, and possibly wrecking the future happiness of their child? And should the possibility of mental defect be mentioned if there is a strong likelihood that it will not occur?

(b) 47,XXX. Here again there is a possibility of mental defect, or at least of some impairment of the mental capacity. Should the parents be warned of this possibility?

(c) 47,XYY. This is perhaps the hardest problem of all, because there is as yet no overall statistical picture of the life-pattern of the 47,XYY individual. While it seems likely that some of these will indulge in antisocial behaviour which may give their parents great worry and concern, it is probably true that many will seem to be perfectly normal. The parents may be aggrieved if they are not given warning and the child is difficult; but also if they are warned, and it is behaviourally normal.

In view of these difficulties, it is hard to avoid the conclusion that in most cases the parents should *not* be told. Probably the best course would be to inform the family physician and to advise him not to tell the parents unless circumstances appear to warrant it.

Is the screening of neonates for chromosomal abnormalities itself justifiable? On scientific grounds it almost certainly is; only thus can an unbiased sample of (for example) XYY individuals be detected at birth, followed through, and thus add to our knowledge of what the statistical outcome for such a group of persons will be. But there seems to be a much less strong case on purely medical grounds. The author feels (although he may be charged with scientific obscurantism) that there is little advantage to be gained by early diagnosis of most sex chromosome disorders; against the knowledge of the likely outcome, has to be set the danger of causing much unnecessary unhappiness to both child and parent. "Sufficient unto the day is the evil thereof." The knowledge that a child is unlikely to be able to have children and may not grow up to be very bright mentally will not make the child or its parents very happy; in fact it may blight what might have been a happy childhood. The knowledge that a child may grow up with an aggressive and difficult personality will not prevent that personality developing; but if it does not develop, much unnecessary anxiety will have been caused.

While the screening of neonates for sex chromosome abnormalities is easy and cheap, and could be done in any reasonably-equipped pathological laboratory, so that the problems involved in whether or not to do it are moral and ethical as much as economic, the full chromosomal analysis of large numbers of newborns would be both time consuming and expensive. Both the setting up and the harvesting of the cultures are normally done by hand, and would be difficult (though not impossible) to automate; but even more time-consuming is the analysis of the chromosome preparations. The

author once calculated that the cost of the preparation of a karyotype in his laboratory was about £10, of which the greatest part was attributable to the technician-hours involved. In a large laboratory this figure could no doubt be greatly reduced, but karyotyping will always remain an expensive exercise. Work has been going on for some years to try and computerize the actual karyotype analysis, but progress on this difficult problem has been slow, and advances in technique (such as the fluorescent and Giemsa methods) introduce new complications which will prolong the period before computers can take over the job from technicians (if indeed they ever can, except for preliminary screening). If there were great economic and social advantages to be gained from automation of chromosome analysis, it would no doubt be introduced sooner or later; but in a world where surplus money can be easily and quickly utilized in the amelioration of social conditions and the saving of life in underdeveloped countries, a sophisticated technique, however fascinating and academically excellent, will only be worth full financial backing if there are great benefits to be obtained. It is dubious whether automatic karyotyping can offer such benefits.

It is probable that the present facilities for chromosome analysis in countries such as Great Britain and the U.S.A. are adequate to carry out such chromosome analysis as is genuinely clinically necessary. Analysis is only really called for if (a) the diagnosis cannot be made without it and/or (b) it helps to decide some future course of action. There is, for example, no need to analyse the chromosomes in a case of Down's syndrome (which is easily diagnosed without) except either for research purposes or to give guidance to the parents or other members of the family. In fact the main function of chromosome analysis must in the long run be in relation to eugenics, and the giving of genetic counselling.

There has been a tendency, since the study of human chromosomes first became fashionable, to refer all sorts of obscure congenital conditions for chromosome analysis. This was quite justifiable when little was known of human cytogenetics. But now that most of the major clinical conditions which are regularly caused by chromosomal abnormalities are well defined, referral is only justified if there is a reasonable possibility of an underlying chromosomal defect. Abnormalities of the sex chromosomes and of the autosomes require to be considered separately in this context.

Abnormalities of the sex chromosomes may be legitimately suspected in a variety of individuals whose sexual development is abnormal; in particular in cases of intersexuality and infertility; and

in amenorrhoea in women, either with or without any degree of mental defect. Finding the particular stigmata of classical Turner's or Klinefelter's syndrome will of course suggest analysis straightway, but other less obvious clinical syndromes (such as the gonadal dysgenesis without dwarfing which is found in persons with an X short arm isochromosome) may demand chromosome analysis for their clarification. Preliminary screening for sex chromatin body and Y-body will usually foretell what will be found on full chromosome analysis.

Abnormalities of the autosomes, on the other hand, virtually all involve the duplication or deficiency of a mass of chromosomal material containing many genes. This almost always leads to obviously detectable physical abnormalities; mental defect is almost invariable, and physical defects, particularly in the head region, are common. So it is a waste of time to refer a patient for chromosome analysis (with a view to finding an autosomal disorder) unless they have some degree of mental defect combined with physical defects, including abnormal dermatoglyphics. Mental normality can be taken as ruling out most detectable autosomal disorders. Nor is it worth referring patients whose only abnormality appears to be due to a single gene of large effect, whether dominant, recessive, intermediate or sex-linked. A condition caused by a single gene will not result from a chromosomal abnormality, which must by its nature involve many genes.

However, mental defect combined with physical abnormalities is very far from being diagnostic of a chromosomal disorder; mental subnormality is commonly accompanied by various physical abnormalities. Observations on inmates of institutions for the mentally subnormal have shown that apart from frank Down's syndrome and abnormalities of the sex chromosomes, detectable chromosomal abnormality is rare and accounts for only a small percentage of the mentally defective population. Apart from the clinically recognizable chromosome disorders which have previously been described, disorders of the autosomes are rare and sporadic.

2. *The Value of Cytogenetics in Genetic Counselling*

Carter (1970) has summarized concisely the role of chromosome analysis in genetic counselling. Most children with chromosomal anomalies are affected as a result of a fresh mutation and have no affected relatives. This applies particularly to the sex chromosome disorders such as the 45,X, 47,XXX, XXY and XYY syndromes, which almost always arise *de novo* as a result of a non-disjunctional

event. The same applies to most autosomal abnormalities, but in the rare cases where this is not so, cytogenetic analysis is important in genetic counselling.

Down's syndrome is the commonest and most important autosomal example, and well illustrates the place of cytogenetics in genetic counselling. Most cases, as already described, are sporadic, and arise from non-disjunction, commonly in an older mother. Some 3% arise through an unbalanced translocation which is detected on chromosomal analysis, and in about half of these one or other parent, usually the mother, carries a balanced translocation. A certain proportion of cases arise through inevitable non-disjunction in a mosaic parent, again usually the mother. The proportion is not known but is probably small.

In order to give the best estimate to the parents of the risk of having another affected child, cytogenetic analysis is desirable, especially if the mother is young (under 30). If the patient is a standard trisomic mongol, and no chromosomal abnormality is found in either parent, the risk of recurrence is small, perhaps only twice that for a mother of the age in question (Carter and Evans, 1961; Hamerton *et al.*, 1961). If a translocation is found in the patient, and in one of the parents, the risk is greater (see Chapter 11). The risk varies with the type of translocation and the parent affected. For D/G translocations the risk of recurrence may be 1 in 5 or 6 if the mother is affected and half this or less if the father is affected. For the rarer G/G translocation, the risk of recurrence is not so well known, but is probably less than 1 in 10 (Hamerton, 1966) with transmission commoner through an affected father than an affected mother (Penrose and Smith, 1966). If either parent is found to be a mosaic for normal and trisomic cells, the risk of transmission is also greater, but will differ in every case, and cannot be assessed from the proportion of trisomic cells in somatic tissues, since the proportion in the gonad may be different.

There is also a good case for performing cytogenetic studies on both parents and child where the child has a structural abnormality of the chromosomes, such as an unbalanced translocation or a deletion (as in the cri-du-chat syndrome). Here again the empirical risk of recurrence when a parent shows a balanced translocation is not well known, but a general estimate of 1 in 5 to 1 in 10 when the mother is affected has been given by Lejeune *et al.* (1970).

However, the majority of births of children with chromosomal abnormalities cannot be prevented by genetic counselling, since they arise *de novo* through non-disjunction, which cannot be prevented

except on a statistical basis, by discouraging women to have children in the latter part of their reproductive life. Half of all children with Down's syndrome in England and Wales are born to mothers over 35; but these contribute only a tenth of all pregnancies. Nevertheless advice to this effect is easier to give than to take; if the author's mother had taken it, he would not be here to write this book.

The only real hope for the prevention of births of children with chromosomal anomalies, when there is known to be a danger of their conception, lies in the possibility of early diagnosis (by amniocentesis or other means) followed by selective abortion. This subject merits a section of its own.

3. Amniocentesis, and the Antenatal Diagnosis of Chromosomal Anomalies

If the cells of an embryo or fetus can be obtained while it is *in utero,* and chromosome preparations made from them (which will normally involve culture), an antenatal diagnosis of a chromosome disorder can be made. Even without culture, examination for sex chromatin and fluorescent Y-body will allow a fairly accurate diagnosis of the number of X and Y chromosomes present.

The only reliable method of obtaining such fetal cells at present is by amniocentesis; the insertion of a needle into the amniotic cavity, and the withdrawal of amniotic fluid, containing a certain number of fetal cells. This is normally performed through the anterior abdominal wall, and so cannot be done until the uterus rises above the pubic symphysis, which is normally some time between the 12th and 14th week. Vaginal amniocentesis is possible, but seems to lead to more complications.

It should in theory be possible to obtain fetal cells in other ways. For example, Shettles (1971) claims that fetal cells can be obtained from the mucus of the uterine cervix in the second and third month of pregnancy, and that the antenatal diagnosis of sex (and therefore by inference, of sex chromosome aneuploidies) can be made from these cells. But this report is a preliminary one, requiring confirmation. It is also very unlikely that these cells could be cultured, so that they could not be used for karyotyping.

Clearly there is a strong possibility that sex chromosome aneuploidy may be diagnosed without resort to amniocentesis. However this is not of much help in genetic counselling, for it would hardly be justifiable to recommend selective abortion of 45,XO, 47,XXX, 47,XXY or 47,XYY embryos even on firm diagnosis; for they may develop into almost or apparently completely normal individuals. A

diagnosis of 48,XXXX or XXXY might justify selective abortion on the grounds of the high likelihood of mental defect; but these aneuploidies are so rare that they are unlikely to be found in this way.

Selective abortion, therefore, is only likely to be practised where there are autosomal anomalies which are virtually incompatible with normality (I exclude here the sinister possibility that it could be used by parents to select the sex of their children, with all the moral and social implications which might follow); and at present, amniocentesis is the only method by which these can be diagnosed.

The advantages and the snags of amniocentesis have been reviewed by Emery (1970). The main advantage is that the procedure is simple; a needle is passed through the anterior abdominal wall into the amniotic sac, under local anaesthesia, and a small amount of amniotic fluid withdrawn. Greater safety is given to the mother and child if the placenta can be located beforehand (by ultrasonics, or some other method) so that it can be avoided. The principal snags are two. First and foremost is the fact that if selective abortion is to be performed, diagnosis needs to be made as soon as possible. While an early abortion is a fairly straightforward operation, performed *per vaginam,* and usually followed by quick recovery, later abortions have to be carried out by other and potentially more dangerous techniques.

Vaginal termination of pregnancy is usually performed by suction curettage, which can be practised with safety up to about 14 weeks (though some would prefer not to do this after 12 weeks). After the fourteenth week three methods are possible. The first is abdominal hysterectomy (a mini-Caesarian section), less used now than it was because of the danger of the scar rupturing in later pregnancies. The second, now quite widely used, is the intra-amniotic injection of saline and glucose or of urea; the third, and most recent, is the intravenous infusion of prostaglandins with or without syntocinon.

As previously mentioned, amniocentesis is not possible before the twelvth week at the earliest, and becomes progressively easier as the uterus enlarges (it has been estimated that the amount of amniotic fluid is about 50 ml at 12 weeks, 150 ml at 15 weeks, and 450 ml at 20 weeks). When the fluid has been withdrawn it has to be cultured to obtain dividing cells. This takes at least one and sometimes up to six weeks (although it is usually possible to tell whether the culture will be successful earlier than this); and a proportion of cultures fail and need to be repeated. Thus it is unlikely that a result will be available in time for an abortion by suction curettage, and

one of the other methods mentioned above will require to be used.

Another possible disadvantage of amniocentesis is the occurrence of injury to either mother or fetus. The danger to the mother lies in possible haemorrhage, infection, or damage to neighbouring viscera. Fortunately these complications are reported to be very rare. The dangers to the fetus include trauma, infection, and spontaneous abortion. These also seem to be fairly rare. Emery (1970) found no instances in 132 cases where amniocentesis was performed early in pregnancy (before 20 weeks) and the pregnancy allowed to proceed to term. On the other hand, Riis and Fuchs (1966) reported a series of 11 cases where vaginal amniocentesis had been carried out which included three spontaneous abortions and one stillbirth. It therefore appears that abdominal amniocentesis in skilled hands is a fairly safe procedure for both mother and fetus, while vaginal amniocentesis may be risky.

Amniocentesis is usually, and best, carried out between 13 and 16 weeks (Emery, 1970) and 5-10 ml of amniotic fluid are withdrawn. Many analyses may be carried out on this fluid, including biochemical estimations and histochemical staining of the contained cells, and these can shed light on various genetically-determined disorders, as well as allowing the determination of the ABO blood group of the fetus. However, from the point of view of this book, consideration will be limited to cytogenetic analysis of the amniotic fluid cells. These cells have been shown to be of fetal origin (although it is not yet proven whence they originate within the fetus; skin and amnion seem most likely). They can be tested directly for the presence of sex chromatin bodies and fluorescent Y bodies; and a full chromosome analysis can be made after culture.

(a) Analysis of sex chromatin bodies. This is not always easy because the number of cells may be very few, or the nuclei distorted or pycnotic. Nelson and Emery (1970) found that sex determination on the basis of the sex chromatin body could only be performed on two-thirds of specimens; but that given a satisfactory specimen, prediction was 90% accurate.

(b) Analysis of fluorescent Y-bodies. It is to be expected that fluorescent staining of the amniotic fluid cells will show up a fluorescent Y-body in a proportion of cases when the fetus is male; there is as yet insufficient data to tell how much information this will add to studies of sex chromatin; but presumably it will allow greater accuracy of sex determination when taken in conjunction with assessment of presence or absence of sex chromatin; at any rate, in those cases where the material is satisfactory. But where the

material is unsatisfactory for sex chromatin analysis it is likely to be unsatisfactory for fluorescent staining also.

The main purpose of such antenatal sex determination will be in the possible prevention of various genetic disorders which are X-linked; thus it has been suggested that it might be performed in such a condition as haemophilia; all male fetuses of a women who was a carrier of the gene could be aborted, thus preventing the birth of affected male haemophiliacs. Whether such a procedure is socially and ethically acceptable is highly debatable; even more debatable is the possibility (already mentioned) that parents might utilize the procedure of amniocentesis followed by selective abortion in order to obtain children of the desired sex.

(c) Chromosome analysis. In order to obtain chromosome preparations, the amniotic fluid cells have to be cultured. Methods have been described for this (Steele and Breg, 1966; Nelson and Emery, 1970). Because the number of cells present may be small, it may, as mentioned earlier, take up to six weeks of culture to obtain adequate numbers of dividing cells for analysis.

The main object of chromosome analysis is in the management of high risk pregnancies, such as those in which one parent, usually the mother, is the carrier of a balanced translocation, of which the commonest is the D/G variety. Where the risk of an affected child is greater than 1 in 20 (as it probably is in most such translocations) amniocentesis is well justified, even if abortion by abdominal hysterotomy is eventually necessary. Another possible indication might be a pregnancy in an older mother, with the possibility of the birth of a child with Down's syndrome or some other trisomic condition. It will be recalled that the risk to the mother over 40 of having a mongol child may be of the order of 1/100 down to 1/50. It is a nicely balanced point whether the risk of having an affected child outweighs the risk to the fetus of amniocentesis; especially if the child in question is the first and much wanted product of a late marriage. The author feels that amniocentesis in such cases is probably not justified in the present state of knowledge, as it is still in the research and development stage; but if centres were to be established where the procedure was carried out regularly by highly-skilled staff, it would probably be warranted. A demand certainly exists, particularly from the well-educated older mother with some medical knowledge.

4. The Value of Cytogenetic Studies as an Index of Mutagenesis

This may prove in the long run to be the field in which

cytogenetics can make the greatest contribution to human welfare. The human race, in its struggle for social and material advancement, is continually polluting its environment with products of its activity which may be in some way beneficial, may be harmful, or which may be mere useless byproducts. A number of these products are mutagenic; radiation from diagnostic radiography or from the explosion of nuclear devices are notable physical mutagens, while a whole host of chemical mutagens are being produced, whose biological significance is as yet unclear (Chapter 13).

The danger of all mutagenic agents is, of course, a long term danger. Small changes are induced in the chain of DNA; deletions, alterations in nucleotide sequence, and so on, which may (and probably will) have no effect on the individual in whom they occur. But where they occur in the germ cells there is a danger to future generations. This, as is well known, is particularly the case where recessive mutations are occurring. A single point mutation may and usually will have no effect on an individual if only present in one chromosome of a pair, since the other normal member of the pair has a normal gene at the site. But an individual with two homologous chromosomes, each of which is mutated at the same site, may be affected by a troublesome or fatal enzyme deficiency or other genetic defect. Such homozygous individuals are most likely to be conceived as a consequence of the intermarriage of descendants of the individual in whom the mutation originally arose. This may not occur for many generations after the mutation has occurred, but as time goes on and the descendants of the original individual multiply, it becomes increasingly likely. After ten or more generations have passed, affected individuals are likely to appear with increasing frequency. So the harm that is being done to germ cells by our present activities is likely to be manifested not immediately but in several hundred years' time.

It is clear that any potentially mutagenic agent requires to be screened as closely as possible before the public are exposed to it. This is fairly straightforward for physical mutagens, and in particular for ionizing radiations. Exposure to these agents can be accurately assessed by physical means, by the direct measurement of dosage received (as is now routine practice for all persons who are regularly exposed to such hazards). It is well known from classical experiments in radiation biology (Chapter 8) that the visible effects produced on chromosomes by radiation are more or less directly proportional to the dose received, so that visible chromosome mutations could well be used as a measure of dose received. This is not necessary for

ionizing radiation, since simpler methods are available (although cytogenetic analysis could still be used retrospectively to estimate amount of radiation received, if no other information were available). But it could become very important in assessing the mutagenic properties of various chemical (and biological) agents, since there is no other direct method of estimating the amount of genetic damage which they may be causing.

The technique for assessing the mutagenicity of chemical and biological agents by the amount of chromosomal damage which they cause are still new and underdeveloped. The difficulties in any such assessment have been discussed in Chapter 13; they include the variability inherent in any biological experiment, and the difficulty of drawing conclusions about *in vivo* events from *in vitro* experiments. Nevertheless, satisfactory techniques for estimation of *in vivo* mutagenicity have been evolved and will no doubt be much improved with experience; and it is likely that they will be able to resolve satisfactorily the arguments about the mutagenic effects of such substances as the cyclamates, or LSD-25, and to present governments with a rational basis for legislation on the use of such substances.

5. The Value of Cytogenetic Studies in Cancer Treatment and Research

Cytogenetic studies in relation to cancer have already been considered in some detail (Chapter 13); but the topic can be summarized by saying that while it seemed at first, during the earliest studies of the chromosomes of tumours, that such studies would not prove particularly helpful since findings were so variable, there now seems to be gathering evidence that definite patterns of chromosomal behaviour occur during the evolution of tumours (including the leukaemias). Knowledge of the subject is still in a very early stage, and with the advent of the new techniques for chromosome identification and for the hybridization of cells, there is every hope that it will advance very rapidly in the next decade or two.

Cytogenetic studies are the only way in which the genetic material of the cell can be actually visualized and localized. In the long run they may prove the best way of assessing the genetic nature of a particular tumour, and of assessing both the prognosis and the best method of treatment. At present the value of cytogenetic studies for prognosis is very limited, although it has, for example, been shown that chromosomal studies can give information about the prognosis in some varieties of leukaemia. But as more information is gathered in, it is probable that chromosomal analysis will afford much useful

information about the nature of individual tumours. As in all biological sciences, it is necessary to have a good knowledge of the normal condition before the pathological one is understood. Basic knowledge of the normal human chromosomes is still at a very rudimentary stage as regards their genetic function, and until more is known about gene localization on chromosomes it will not be possible to understand the significance of particular patterns of chromosome distribution in particular tumours.

IV. HUMAN CYTOGENETICS IN PERSPECTIVE

The opening up of research on the human chromosomes which was made possible by the work of Tjio and Leven and of C. E. Ford and Hamerton in 1956 raised great hopes that rapid and useful advances in human genetics would follow. An army of scientists from many countries entered the field, and a vast and exponentially increasing number of publications have appeared. Has all this expense and effort paid off in terms of achievement?

It must be admitted that the cost-effectiveness of the vast sums of money which have gone into human cytogenetics is not at present very great. The contributions to medicine have been considered above, and they do not in aggregate amount to very much, even after 15 years of research. If the equivalent amount of money had been spent in the provision of food and equipment for underdeveloped countries, many lives could have been saved, and much temporary happiness promoted. But this is a very short-sighted view. The contribution of all this work to our store of fundamental biological knowledge has been enormous. It is easy, and nowadays popular, to criticize the accumulation of knowledge for its own sake. But it is always impossible to forecast when knowledge which appears to be pure (and useless) may find an application. There are a number of pointers to the future use of knowledge of the human chromosomes, which indicate that the information which has been so hardly gained will find applications in due course.

If, as has just been suggested, the money which has been spent on fundamental research on the human chromosomes had been diverted to saving life, what would have been the gain to mankind? What matters is not mere survival, but the quality of life. The contributions of cytogenetics to human genetics promise, in the long run, to add greatly to the quality of life lived by future generations. The money spent on it has been, by any criterion, well invested.

§15§

Gene Loci on Chromosomes

J. H. RENWICK

Reader in Human Genetics and Population Teratology, London School of Hygiene and Tropical Medicine, London, England

Many aspects of chromosomes have been considered, and in this final chapter it would seem appropriate to summarize briefly what is known about the gene loci carried by specific chromosomes. The methods using data from human pedigrees are summarized by Renwick (1971b). The methods employing cell hybrid techniques depend upon the concurrence of the loss of a human chromosome pair with the loss of a specific protein, usually an enzyme. A gene locus concerned with the synthesis of that enzyme can then be confidently assigned to that chromosome pair. Such methods are summarized by Ruddle (1971) whose team has successfully assigned many of the gene loci indicated in Fig. 59. The manikin in this figure consists of lines of lengths that are proportional to the lengths of the 22 pairs of autosomal chromosomes. The positions of the numbers indicate approximate positions of the centromeres.

The hybrid-cell methods have not yet been adapted to yield estimates of distances between gene loci. These are given by the pedigree methods. In Table 12, the distances that have been estimated between certain of the autosomal loci shown in Fig. 59 are given, together with some distances between loci that are known to be on the *same* chromosome pair because of their joint segregation properties in pedigrees but which have not yet been assigned to any particular pair. The distances that are given are the average of the male and female distances. The unit of map-distance that is used here is the centimorgan (cM). This is equal to one-hundredth of a morgan, where one morgan is the length of the chromosome map that experiences, on average, one cross-over event per meiosis. Table 12 also depicts the map of the X-chromosome. The ordering of the loci is only one of the most probable orderings and many of the map

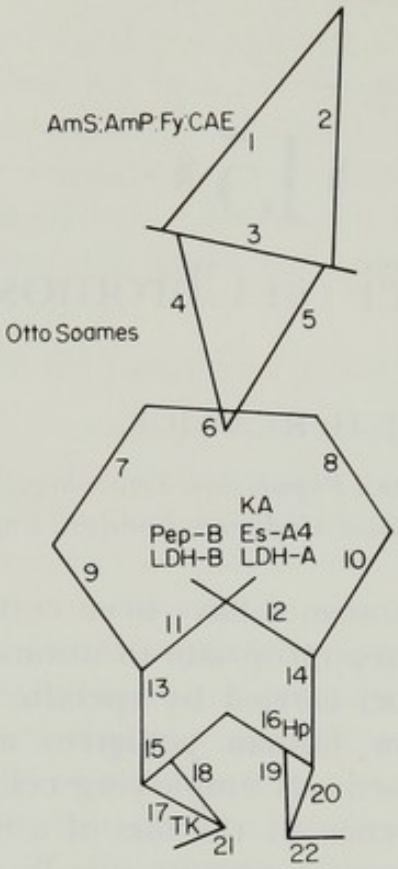

Fig. 59. Man's map maps man. Representation of the 22 human autosome-maps of lengths proportional to the physical lengths of the corresponding chromosomes at mitotic metaphase. The map-position of the centromeric-point is indicated roughly by the position of the numerical designation of each map. Assigned (and probably assigned) loci are noted. This manner of presentation is unfortunately not amenable to the display of distances.

Map–1 (refs 3, 11, 14, 15, 22, 23, 27, 29, 41)	AmS (Amy–1)	Amylase, salivary
	AmP (Amy–2)	Amylase, pancreatic
	Fy	Duffy blood group
	CAE	Cataract, total nuclear
Map–11 (refs 16, 26, 33, 50)	KA	Killer antigen
	Es-A4	Esterase A4
	LDH-A	Lactate dehydrogenase (A locus)
Map–12 (ref. 31, 49)	PepB	Peptidase-B
	LDH-B	Lactate dehydrogenase (B locus)
Map–16q (refs 30, 19, 28)	Hp	Haptoglobin
Map–17q (refs 1, 9, 17, 18, 24, 33)	TK	Thymidine kinase

See Addendum B, p. 357

Table 12 Human linkage groups*

Linkage group†	Loci	References since ref. 28
1	1qh : Fy : Cae : : AmP : AmS (8, 0, 20, 0)	11, 14, 15, 22, 23 (and see Addendum B, p. 357)
ABO	Np : AK : : ABO (0, 13)	–
MNS	Tys : MNS (4)	–
Rh	PGM1 : : Rh : EL1 : : PGD : : PepC (27, 3, 21, ?)	2, 21, 25, 27, 39, 40 (and see Addendum B, p. 357)
Se	Dm : Se : :Lu (4, 13)	45, 47, 48
Hb	Hbβ : Hbδ (0)	–
Gm	γG2 : γG3 : γG1 : γA2 : : Pi (0, 0, 0, 25)	7
Tf	Tf : : E_1 (14)	–
Gc	Alb : Gc (2)	–
HL-A	P : : HLA_2 : HLA_1 : : PGM3 (1, 21)	6, 43, 51
IDH	IDH : : MOR-2 (?)	36
X		4, 5, 8, 10, 12, 13, 37, 42, 44, and esp. 34, A12

−27 −17 0 11 24 32 46 52 56 60 cM from Xg

rs : : oc.alb : : Xg : : ich : : α–GAL : HGPRT : : h′A : G6PD : deut : prot : : md : : h′B : : PGK (6, 4, 4, 30)

*Linkage groups 1 and X and those not yet assigned to specific chromosome maps. In no linkage group is the ordering fully established and the ordering indicated here is merely one of the most probable. The estimates of map distances are mutually consistent and are therefore conditional on the ordering indicated. They are given in centimorgans (cM) and are the average of male and female values. The precision of these estimates varies widely but is poor in the main, especially for long intervals. Information on it is in most cases obtainable from the references quoted.

†To avoid the confusion generated by the double numbering system in the mouse, we designate the human unassigned linkage groups by the name of the first polymorphic marker to be described in that linkage group.

distances given have poor precision. Edwards (1968) and Sanger and Race (1971) summarize the present position in X-chromosome mapping. References are given by Sanger and Race (1970).

Additional gene loci are being placed and linkages are being established between pairs of loci at a reasonably rapid rate, hence these maps will be partly out of date even before they are published.

Apart from its intrinsic interest, this type of information concerning our chromosomes occasionally has a medical application in assisting the pre-natal diagnosis of certain genotypes that would later produce a disease phenotype. This diagnosis can occasionally be early enough so that selective abortion is an option open to the parents. For example, in occasional pedigrees the secretor status of the fetus as determined on the amniotic fluid can indicate with about 92% security the transmission of the myotonic dystrophy allele from a parent who carries it.

At the purely scientific level the chromosome map information should prove useful in ways that are at present largely unpredictable. In many other species, it has certainly been experienced that such knowledge opens up a large number of opportunities for probing a wide range of biological mechanisms.

Key to Locus Symbols (Autosomal) in Fig. 59 and Table 12.

Locus symbol	*Locus of*
ABO	ABO blood group
AK	Adenylate kinase (erythrocytic)
Alb	Albumin
AmP	Amylase, pancreatic
AmS	Amylase, salivary
Cae	Total congenital nuclear cataract
Dm	Myotonic dystrophy
E_1	Cholinesterase E_1
EL1	Elliptocytosis
Es-A4	Esterase-A4
Fy	Duffy blood group
Gc	Group-specific glycoprotein
γA2	Immunoglobulin heavy chain locus for IgA_2
γG1	for IgG_1
γG2	for IgG_2
γG3	for IgG_3
Hb_β	$Haemoglobin_\beta$
Hb_δ	$Haemoglobin_\delta$
HLA_1	Histocompatibility leucocyte antigen – LA
HLA_2	Histocompatibility leucocyte antigen – 4
Hp	$Haptoglobin_\alpha$
IDH	Isocitrate dehydrogenase (mitochondrial)

KA	Killer antigen
LDH-A	Lactate dehydrogenase-A
LDH-B	Lactate dehydrogenase-B
Lu	Lutheran blood group
MOR-2	Malate dehydrogenase (mitochondrial)
MNS	MNS blood group
PepB	Peptidase-B
PepC	Peptidase-C
PGD	6-phosphogluconate dehydrogenase
PGM_1	Phosphoglucomutase-1
PGM_3	Phosphoglucomutase-3
Pi	Protease inhibitor
Rh	Rhesus blood group
Se	ABH secretor
Tf	Transferrin
TK	Thymidine kinase
Tys	Sclerotylosis

Key to Locus Symbols (X-linked) in Table 12

Locus symbol	*Locus of*
deut	Deuteranopia
α-GAL	α-galactosidase (Fabry's disease, angiokeratoma corporis diffusum)
G6PD	Glucose 6-phosphate dehydrogenase
h′A	Haemophilia-A
h′B	Haemophilia-B
HGPRT	Hypoxanthine guanine phosphoribosyl transferase (Lesch-Nyhan syndrome)
ich	X-linked ichthyosis
md	Duchenne muscular dystrophy
oc.alb	Ocular albinism
PGK	Phospho-glycerate kinase
prot	Protanopia
rs	Retinoschisis
Xg	Xg blood group

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Appendix

Nomenclature and standardisation in human cytogenetics

The main points from the Denver (1960), London (1963) and Chicago (1966) conferences are summarized in this appendix. Readers who wish to study the full texts of the reports of these historically important conferences are referred to the originals, which are included in the reference list under the above titles. They are also quoted in full in Appendices II-IV of Vol. I of Hamerton's "Human Cytogenetics" (1971). The account of the Chicago Conference published by the National Foundation includes reprints of the reports of the preceding two conferences.

I. DENVER CONFERENCE (1960)

A. Proposed Standard System of Nomenclature of Human Mitotic Chromosomes (Extract).

"It was agreed that the autosomes should be serially numbered 1-22, as nearly as possible in descending order of length, consistent with operational conveniences of identification by other criteria. The sex chromosomes should continue to be referred to as X and Y, rather than by a number, which would be an additional and, ultimately, a superfluous appellation.

It was generally agreed that the 22 autosomes can be classified into seven groups, distinction between which can be readily made. Within these groups further identification of individual chromosomes can in many cases be made relatively easily. Within some groups, especially the groups numbered 6-12, including also the X chromosome, the distinctions between the chromosomes are very difficult to make by presently available criteria. However lesser difficulties are encountered in separating chromosomes 6 and X from the remainder of this group. It is believed that, with very favourable preparations, distinction can be made between most, if not all, chromosomes.

It is proposed that the autosomes first be ordered by placing the seven groups as nearly as possible in descending order of size. Within each group the chromosomes are arranged, for the most part, by size. It was desired specifically to avoid the implication that size relationships have been permanently decided in every instance, but it is hoped that the assignments of numbers will be permanently fixed. In those cases where distinction is at present doubtful, final definition of each chromosome can be left until further knowledge has accrued, although an attempt is made to provide a characterization of each."

The report went on to give the characterization of each chromosome group (which is not quoted here since it is now outdated), a table giving the quantitative characteristics (measurements) of the chromosomes according to six different groups of workers, and a table reconciling the nomenclature of different workers. It is not necessary to quote these here at length, but the

following table (Table 13) gives the means of the six sets of measurements as given by Penrose (1960).

Table 13

Means of the six sets of length values for the human chromosomes given in the report of the Denver Conference, expressed in terms of a total haploid length of 1000 units.

Chromosome No.	1	2	3	4	5	6	7
Short arm	41.5	31.6	30.8	16.6	16.0	20.6	18.6
Long arm	44.7	49.1	36.9	45.7	42.2	34.9	31.5

8	9	10	11	12	X	13	14	15
15.3	16.2	13.8	15.0	12.4	19.9	4.8	5.2	4.8
31.2	29.2	30.7	28.5	29.9	35.2	28.9	28.8	27.1

16	17	18	19	20	21	22	Y
12.1	9.0	6.4	9.6	9.3	4.2	3.9	2.0
18.4	20.5	19.2	13.9	12.2	12.4	11.6	15.7

The system of nomenclature proposed at the Denver Conference has been generally accepted, with one exception. It was for some time considered that the chromosome which was trisomic in Down's syndrome was No. 21, the longer of the two smallest pairs of autosomes. However these two chromosomes are very close in size, and recent work on measurement, assisted by individual identification of the members of the two chromosome pairs by fluorescent and other techniques, has shown that the trisomic chromosome in Down's syndrome is actually the shortest autosome (no. 22 of the Denver classification). However, the term "trisomy 21" as a description of the chromosomal anomaly in Down's syndrome has been so much used in the literature that it was felt that it would be confusing to comply with the Denver classification over these two chromosomes, and it has been internationally agreed that the smallest autosome, trisomic in Down's syndrome, should continue to be called No. 21, and the larger of the chromosomes in group G should be called No. 22.

II. THE LONDON CONFERENCE ON THE NORMAL HUMAN KARYOTYPE (1963)

This conference further characterized certain of the human mitotic chromosomes, in the light of work published since the Denver Conference. The main advances are summarized in the following two paragraphs from the report:

"The most important newly recognized features are so-called secondary constrictions and differential patterns of incorporation of isotopically labelled thymidine in specific chromosomes as demonstrated by autoradiography. Secondary constrictions are only seen in certain types of preparations and then in a fraction of the cells.

Variability is observable in the morphology of certain chromosomes. This is especially well established for the Y chromosomes in which great differences in size occur and which are heritable from father to son. Heritable polymorphism has also been observed in satellited chromosomes and probably in No. 16."

III. REPORT OF THE CHICAGO CONFERENCE (1966)

The purpose of the report is summarized in the following paragraph:

"This report considers new means for describing normal chromosomes and deviations from the normal complement. In addition to proposing a system of nomenclature, suggestions are made for the inclusion of publications of genetic and other data which may enhance the significance of chromosome findings. A uniform system of notation is essential if an international human cytogenetics register is to be established. The physical plant required for such an organization would of course include automated methods for data handling, and recommendations are made with this in mind."

The proposed system of nomenclature has now been generally adopted, and because of its importance it is quoted below *in toto*. The remaining parts of the report, concerning data recording, procedures (autoradiography, and automation of chromosome analysis), special studies (population studies, radiation and other mutagens, and abortion studies) and recommendations for improvement in communication and the establishment of a central registry, will not be quoted here since, although important, many of the points are dealt with in the text of this book.

A. Nomenclature Recommended by the Chicago Conference

A system of nomenclature is proposed for the description of the human chromosome complement and its abnormalities, utilizing designations which can be easily understood, written and coded for data retrieval. A list of recommended symbols is given in a table at the end.

B. Numerical Aberrations

In a description of karyotype findings the first item to be recorded is the total number of chromosomes, including the sex chromosomes, followed by a comma (,). The sex chromosome constitution is given next, e.g.

45,X	45 chromosomes, one X chromosome
47,XXY	47 chromosomes, XXY sex chromosomes
49,XXXXY	49 chromosomes, XXXXY sex chromosomes

The autosomes are specified only when there is an abnormality present. Thus, if there is a numerical aberration of the autosomes, the group letter of the extra or missing autosome, followed by a plus (+) or minus (−) sign, succeeds the sex chromosomes designation, e.g.

45,XX,C−	45 chromosomes, XX sex chromosomes, a missing C group chromosome.
48,XXY,G+	48 chromosomes, XXY sex chromosomes, an additional G group chromosome.

The plus or minus sign after a chromosome letter or number indicates that the *entire* autosome is extra or missing. When the extra or missing chromosome or chromosomes have been identified with certainty, the chromosome number may be used, e.g.

45,XX,16–	45 chromosomes, two X chromosomes, a missing No. 16 chromosome.
47,XY,21+	47 chromosomes, XY sex chromosomes, an additional No. 21 chromosome.
46,XY,18+,21–	46 chromosomes, XY sex chromosomes, an extra No. 18 and missing a No. 21.

A question mark (?) is used in the normal way to indicate uncertainty. If it is suspected that a missing or extra chromosome belongs to a particular group, but this is not certain, the question mark may *precede* the group designation or in some cases the chromosome number, e.g.

45,XX,?C–	45 chromosomes, XX sex chromosomes, a missing chromosome which probably belongs in group C.

Another example would describe the karyotype of a sex chromatin positive female with an additional small acrocentric chromosome; this could be written, depending on the amount of available information, as: 47,XX,?G+; 47,XX,G+; 47,XX,?21+ or 47,XX,21+.

A *triploid* or *polyploid* cell should be evident from the chromosome number and from the further designations, e.g. 69,XXY; 70,XXY,G+. An *endoreduplicated* metaphase can be indicated by preceding the karyotype designation with the abbreviation "end", e.g., end 46,XX. If multiplicity of endoreduplications are to be indicated, an Arabic numeral can be used before "end" to indicate this, e.g. 2end46,XX; 4end46,XX.

The method for describing chromosome complements containing structurally rearranged chromosomes, such as dicentrics, acentric fragments and markers, is given later.

1. Chromosome Mosaics

The chromosome constitution of the different cell lines are listed in numerical or alphabetical order, *irrespective of the frequencies of the cell types in the individual studied.* The karyotype designations are separated by a diagonal (/), e.g.

45,X/46,XY	A chromosome mosaic with two cell types, one with 45 chromosomes and a single X, the other with 46 chromosomes and XY sex chromosomes.
46,XX/46,XY	A chromosome mosaic with both XX and XY cell lines.
46,XY/47,XY,G+	A chromosome mosaic with a normal male cell line and a cell line with an extra G group chromosome.
45,X/46,XX/47,XXX	A triple cell line mosaic.

Structural Alterations

The *short arm* of a chromosome is designated by the lower-case letter "p", the *long arm* by the letter "q", a satellite by the letter "s", a secondary

constriction by the letter "h" and the *centromere* by the abbreviation "cen".

Increase in length of a chromosome arm is indicated by placing a plus sign (+) and *decrease in length* by placing a minus sign (−) *after* the arm designation, e.g. 2p+; Bp−; Gq−. When one arm of a mediocentric chromosome, viz. Nos 1,3,19 and 20, is changed this is indicated by placing a question mark *between* the chromosome designation and the plus or minus sign. For example, a No. 3 chromosome with an elongated arm would be designated as 3?+.

The result of a *pericentric inversion* is indicated by p+q− or p−q+, which is enclosed in parentheses and preceded by the abbreviation "inv", e.g. inv (Dp+q−).

A translocation is indicated by the letter "t" followed by parentheses which include the chromosome involved, e.g.

46,XY,t(Bp−;Dq+) or 46,XY,t(Bp+;Dq−)	A balanced reciprocal translocation between the short arm of a B and the long arm of a G chromosome

Translocations involving a sex chromosome and an autosome would be designated as, e.g.

46,X,t(Xq+;16p−)	A reciprocal translocation between the long arm of an X and the short arm of a No. 16 in a female.
46,Y,t(Xq+;16p−)	The same translocation in a male.
46,X,t(Yp+;16p−)	A reciprocal translocation between the short arm of a Y and the short arm of a No. 16.

The remaining sex chromosome is written in its usual position after the chromosome number, and the other sex chromosome which is involved in the translocation is included in parentheses preceding the autosome concerned.

The separation of the chromosomes within the parentheses by a semicolon (;) indicates that two structurally altered chromosomes are present and that translocation is balanced. In a "centric fusion" type of translocation, in which only one translocation chromosome is present, the semicolon is omitted, e.g.

45,XX,D−,G−,t(DqGq)+	45 chromosomes, XX sex chromosomes, one chromosome missing from the D group and one from the G group, their long arms having united to form a DG translocation chromosome.

If, as rarely happens, a small centric fragment is present as well, implying a reciprocal translocation, it could be written as, e.g. 46,XX,D−,G−, t(DpGp)+,t(DqGq)+.

Where a "centric fusion" type of translocation results in duplication of part of one of the chromosomes involved, this could be written as, e.g.

46,XX,D−,t(DqGq)+	46 chromosomes, XX sex chromosomes, one chromosome missing from the D group; the long arm of this chromosome is united with the long arm of a G group chromosome. Since there are four normal G group chromosomes, part of a G is present in triplicate.

When family studies clearly show that a particular chromosome has been inherited from the mother or the father, this may be indicated by the abbreviations "mat" or "pat". For instance, in a family in which a father is carrying a balanced reciprocal translocation, 46,XY,t(Bp−;Dq+), and in which

his malformed son has inherited only one of the two abnormal chromosomes, the son's complement would be written as 46,XY,Bp−pat or 46,XY,Dq+pat, depending on which abnormal chromosome had been transmitted. If the son had inherited both chromosomes involved in the translocation, his complement would be expressed as 46,XY,t(Bp−;Dq+)pat.

Duplicated chromosome structures are indicated by repeating the appropriate designation. Thus 46,XX,Gpss would describe the karyotype of a female in which one of the G group chromosomes has double satellites on the short arm. If satellites appear on a chromosome arm where they are not usually found, this arm should be designated, e.g. 46,XY,18ps, indicating a No. 18 with satellited short arms; 46,XX,Gpsqs, indicating a G group chromosome with both long and short arms satellited. Enlarged satellites are indicated, for instance, by Gs+.

Isochromosomes are designated by the lower-case letter "i" placed after the chromosome arm involved, e.g. 46,XXqi; or if this is presumptive, then 46,XXq?i. This would indicate an isochromosome or a presumptive isochromosome for the long arm of one X chromosome.

Ring chromosomes are indicated by the letter "r" placed after the chromosome involved; e.g. 46,XXr would indicate a ring X, and a ring B would be written 46,XY,Br.

In describing cells damaged by ionizing radiation, chemicals, viruses etc., the system of nomenclature that has been described should be used where applicable. This may not be possible where the cell contains a grossly unbalanced chromosome complement. In such instances the following convention is suggested. The chromosome count in a given cell should include all centric chromosome structures present in that cell regardless of the number of centromeres. Unidentified chromosomes are indicated by "mar" (marker). *Acentric* fragments are *not* included in the count but may be indicated by "ace". *Dicentric* and *tricentric* chromosomes are counted as one body and indicated by "dic" and "tri". As an example, a cell derived from a normal female with a total of 48 centric chromosome structures, one missing F group chromosome, a *dicentric* and an *acentric* fragment, as well as two unidentified marker chromosomes, would be written as: 48,?X?X,F−,dic+,mar1+,mar2+,ace*. If necessary an asterisk (*) following a chromosome designation may be used to draw attention to an explanation in the text.

If *other designations* are needed for special conditions, they should, whenever possible, be taken from the first three letters of the word required, used in lower case, clearly defined and placed immediately before or after the chromosome symbol or the bracketed chromosome designation to which they refer. If single letters are to be used for special designations, they should be in lower case and should not duplicate the capital letters A to G, X and Y or those lower case letters representing other structures. Lower-case letters which are easily confused with numerals, such as "l" or "o" should not be used. Subscripts and superscripts should be avoided since they are easily written incorrectly, not readily handled by computers and difficult to set in type.

In regard to *abnormal chromosomes described for the first time*, it was suggested in the Denver Report that such chromosomes be named after the laboratory of origin. Instead, it is now proposed that the chromosome be described according to its morphology, by use of the shorthand nomenclature recommended. The only exception to this, for historical reasons, is to be the Ph_1 chromosome first described by Nowell and Hungerford (1).

The terms describing abnormalities of chromosome number, such as aneuploid, heteroploid, etc., are often used in a variety of ways. Levan and Müntzing (2) have restated the original definitions of these and other terms and commented on usage.

A further problem that often arises in chromosome nomenclature is that of defining the position of the centromere. Such terms as telocentric and submetacentric are sometimes musused. Levan *et al.* (3) have proposed a standardized nomenclature defining the centromeric position in terms of the arm ratio.

TABLE OF NOMENCLATURE SYMBOLS

A-G	the chromosome groups
1-22	the autosome numbers (Denver system)
X,Y	the sex chromosomes
diagonal (/)	separates cell lines in describing mosaicism
plus sign (+) or minus sign (−)	when placed immediately after the autosome number or group letter designation indicates that the particular chromosome is extra or missing; when placed immediately after the arm or structural designation indicates that the particular arm or structure is larger or smaller than normal
question mark (?)	indicates questionable identification of chromosomes or chromosomes structure
asterisk (*)	designates a chromosome or chromosome structure explained in text or footnote
ace	acentric
cen	centric
dic	dicentric
end	endoreduplication
h	secondary constriction or negatively staining region
i	isochromosome
inv	inversion
inv(p+q−) or inv(p−q+)	pericentric inversion
mar	marker chromosome
mat	maternal origin
p	short arm of chromosome
pat	paternal origin
q	long arm of chromosome
r	ring chromosome
s	satellite
t	translocation
tri	tricentric

repeated duplication of chromosome structure symbols

Reference

(1) Nowell, P. C. and Hungerford, D. A. (1961). Chromosome studies in human leukemia. II. Chronic granulocytic leukemia. *J. nat. Cancer Inst.* 27, 1013-1015.
(2) Levan, A. and Müntzing, A. (1963). Terminology of chromosome numbers. *Port. Acta biol.* 7, 1-16.
(3) Levan, A., Fredga, K. and Sandberg, A. A. (1964). Nomenclature for centromeric position on chromosomes. *Hereditas* 52, 201-220.

Addendum A

This addendum includes some of the more interesting and important observations which have been encountered since this book was written, and which are especially relevant to what has been said in the text. The addendum is not intended, for obvious reasons, to be exhaustive.

Chapter 2

Terminology

The Fourth International Conference on Standardization in Human Cytogenetics at Paris, 1971, has made certain further recommendations on terminology. Some important points are:

1. The chromosome which is trisomic in Down's syndrome, and which is both the shortest and the most fluorescent of the G group chromosomes will be called No. 21.

2. The chromatin of the sex chromatin (Barr) body and of the fluorescent Y-body should be referred to as X-chromatin and Y-chromatin respectively, in order to avoid ambiguity.

3. Recommended terminology for chromosomal banding is as follows:

Quinacrine bands	Q-bands
Giemsa bands	G-bands
Centromeric banding	C-bands
Bands showing reversed contrast	R-bands

Further observations on the nature of chromosome banding

Jones and Corneo (1971) have studied satellite-I and -II DNA in human chromosomes, and have shown that both these DNA fractions are slightly lighter than homogeneous main band (HMB) DNA. Autoradiographic studies on the hybridization of satellite-II with human chromosomal DNA show that it is mostly taken up by the juxtacentromeric heterochromatin of chromosomes 1, 16, and an as yet unidentified C group chromosome, and that the areas involved represent 1.9-2.4% of the total chromosomal area.

Saunders *et al.* (1972) have described a new satellite DNA which is slightly heavier than HMB, and which can be located by hybridization techniques mainly in the juxtacetromeric heterochromatin of chromosome 9, although smaller amounts seem to be located on almost all the chromosomes. The satellite has a high denaturing temperature and so may be inferred to be rich in GC and low in AT base pairs.

Bobrow *et al.* (1972) have described a new Giemsa staining technique which they call the Giemsa-11 technique, since the main feature is staining at pH 11. This gives an intense red colour to the centromeric heterochromatin of chromosome 9, and also stains regions in the short arms of chromosomes 7 and 17 and all the acrocentric chromosomes (including the satellites), and in the long arms of chromosomes 1, 5, 10 and 20. The centromeric staining on No. 9 is so

intense that it can be seen in the interphase nucleus. The authors consider that this technique shows up the satellite DNA described by Saunders *et al.* (1972); the reason for the specific staining is not yet known.

De la Chapelle *et al.* (1971) have clarified the effects of various treatments which have been claimed to denature chromosomal DNA, by staining chromosomes with acridine orange. This stain gives a red fluorescence with single-stranded DNA and with RNA, and a green fluorescence with double-stranded DNA. This method shows that DNA is *not* denatured by fixation with 3/1 acetic/ethanol, by treatment with RNase or 0.2M HCl, or by air-drying the chromosomes at 40° C. Chromosomal DNA *is* denatured by treatment with 0.03-0.7 NaOH for 2-10 min or by boiling in 0.015M NaCl, 0.0015M trisodium citrate at pH 7. After such treatment, heating at 60°-67° C for 1-16 h in 0.9M NaCl, 0.09M trisodium citrate causes partial renaturing. All the chromosomal regions potentially brightly fluorescent with QM fluoresced brightly after this treatment, confirming the view that brilliant fluorescence is confined to chromosomal areas containing repetitious and extensively-renaturing DNA. Such areas clearly may contain different proportions of the main DNA bases, as the previously quoted work on the satellite DNAs implies.

This work also seems to indicate that the bands of lower fluorescence, and the bands seen with the ASG technique, are not related to DNA base specificity. The fact that good banding can be seen after treatment with trypsin or pronase seems to indicate that the banding is related to differences in the DNA-protein complex and that the production of banding patterns is related to the removal of protein. This interpretation is also favoured by Ridler and Ohara (1972). They studied chromosomes, which had been fixed with acetic-ethanol, under the electron microscope, and found that trypsin treatment led to augmentation of band contrast and, at higher power, to the production of a complex network of chromosomal fibres; and also consider that since apparently identical banding patterns are produced by proteolytic enzymes, protein-extracting solutions, and hot salt solutions, the banding must be mainly determined by chromosomal proteins.

Crossen (1971) has shown that Giemsa banding can be obtained on absolutely fresh slide preparations by simple incubation in buffers, whereas after a week it is necessary to treat the chromosomes with NaOH to produce banding; and Ansede (1972) has found that banding after trypsin treatment is improved if the slides are kept for a week. These findings suggest that fixation may denature the chromosomal *protein*, which then progressively hardens with time.

In short, brilliantly fluorescent chromosomal regions probably contain repetitious DNA with a base composition which may be different from that of HMB; while the bands of lower fluorescence are probably related to differences in the DNA-protein complex at the sites where they occur.

Recent findings consequent on the introduction of the fluorescent and pretreatment-Giemsa staining techniques

Many recent advances have been summarized in a review article by Pearson (1972).

The brilliant fluorescence shown by the long arm of the Y chromosome has been utilized in a number of ways. The use of the Y-body in screening cells for sex determination is being widely exploited (e.g. Robinson, 1971, who studied the incidence of fluorescent Y-bodies in the buccal mucosal cells of neonates,

and found that the average incidence of cells showing Y-body fluorescence was 5.4% in 46,XX females, 67.4% in 46,XY males, and 82.9% in the one 47,XYY male found in this series). Pearson and Collacott (1971) have shown that the Y chromosome has a characteristic paranucleolar position in the interphase nucleus. It has been shown that individuals with unusually large (or even normal-sized) Y chromosomes may exhibit fluoresent "drumsticks" in their polymorphonuclear leucocytes (Ricci *et al.*, 1971, Lamborot-Manzur *et al.*, 1971). Manolov *et al.* (1971) have characterized the fluorescent bands found in the long arms of typical Y chromosomes, naming them as q1-q4, and this has been utilized to describe duplication in a Yq+ chromosome (Knuutila and Gripenberg, 1972).

Y-body fluorescence has also been used in the sexing of amniotic fluid cells. Mukherjee *et al.* (1971) have shown that identification of the Y-body is not always possible in uncultured amniotic fluid cells; however in cultured amniotic fluid fibroblasts the body can easily be seen. The proportion of cells showing a fluorescent Y-body varied from 75-84%. X-chromatin also fluoresced in 44-74% of cultured female cells, the proportion depending on cell density in the culture (see p. 145), but could readily be distinguished from Y-chromatin.

X- and Y-chromatin can also be distinguished after fluorescent staining of cells from hair roots (Goday *et al.*, 1971), so that the fluorescent technique alone is clearly adequate for the determination of cell sex, given the right cells.

The new chromosome banding techniques have found wide clinical application; amongst some outstanding findings are the following:

1. Four patients have been described who manifested a distinctive trisomy-8 syndrome (Caspersson *et al.*, 1972). Features were mental retardation (without severe subnormality), clinodactyly and other skeletal defects, concomitant strabismus, and abnormalities of the great vessels (in two). Dermatoglyphic abnormalities were found in two cases studied (Penrose, 1972; Tuncbilek *et al.*, 1972). Most previously reported cases of trisomy-C have been found to be mosaics (p. 250), but two of these four cases appeared to be pure trisomics. The mean maternal age in this small series was 32.2. The syndrome seems to be less deleterious than any other autosomal trisomy, including Down's syndrome.

2. It is clear that the origin of triploidies can often be demonstrated by fluorescent studies of the chromosomes. Jonasson *et al.* (1972) have shown that in two of three triploid embryos studied, the triploidy must have arisen through non-reduction at the first meiotic division in the mother (p. 251).

3. Sergovitch (1971) has described seven new cases of trisomy-G without Down's syndrome, which might previously have been thought to be instances of trisomy-22 (p. 249). Fluorescent studies showed that the group was chromosomally heterogeneous; they were also, as might be expected, clinically heterogeneous.

4. Mark *et al.* (1972) have shown that the G group chromosome which is lost in human meningeiomata is almost always No. 22.

5. Allderdice *et al.* (1971) have demonstrated a possible spreading effect in a 14:Xq unbalanced translocation identified by fluorescence. The patient was effectively trisomic for chromosome 14, but the symptoms were very mild, and this was attributed to spreading of the inactivation of the translocated portion of the X chromosome to involve the trisomic chromosome 14.

Numerous papers have described how the components of various deletion and translocation chromosomes may be identified using the new techniques.

Non-random chromosome association

Further support for the idea that mammalian chromosomes are not randomly arranged in cells comes from observations by Heneen and Nichols (1972) in the Indian muntjac, *Muntiacus muntjac*, which has a very small somatic chromosome number. Cells with homologous chromosomes adjoining were found more frequently than expected, and if no homologues were adjacent, symmetrical radial arrangements based on homology or proximity in size were found. Relevant factors might be somatic pairing, symmetrical spatial arrangements based on homology or size, or association of heterochromatin.

Chapter 7

Xg and X-inactivation

Ducos *et al.* (1971) have studied a human twin chimaera pair, both members of which had a mixture of cells of two different blood groups, O and AB. The O cells were all found to be Xg(a+) and the AB cells Xg(a−), suggesting that the antigen was made on the ancestral red cells, and is therefore capable (despite what is said on p. 149) of giving information about X-inactivation. This strongly supports the supposition that inactivation of a structurally-normal human X chromosome is *not* complete.

Chapter 9

Chromosomal abnormalities in abortuses

Carr (1971) has written a recent review. It seems clear that the incidence of chromosomal abnormalities in early abortuses is considerably higher than in later ones.

Alberman *et al.* (1971) found that 845 mothers who had had spontaneous abortions had received significantly more irradiation during their lives than a control group, especially where the abortus had a chromosomal abnormality (which was in about a quarter of cases). They concluded that radiation increases the risk of a chromosomally abnormal conceptus, although most such conceptuses are lost early in pregnancy.

Chapter 10

C. E. Ford (1970) has reviewed the subject of sex determination in relation to cytogenetics in man and mammals. He concludes that sex in man is determined by the presence of a Y chromosome (male) or its absence (female), but the presence of genetic loci on both the long and the short arms of the X chromosome in double dose is necessary for the development of mature, functional ovaries. The masculinizing locus on the Y chromosome may lie on the short arm, and there is as yet not enough evidence to determine whether it acts directly or by the activation of another locus on an autosome. Turner's syndrome is a genetic deficiency syndrome attributable to the absence in all cells of a segment of the short arm of the X chromosome which is basically homologous to a corresponding segment in the Y chromosome. Part of the evidence for this is that in a few cases of males with 46,XX karyotype, it has

been shown that the father of the propositus has failed to pass on an Xg(a) locus to his son, suggesting that the presence of testicular tissue is due to an exchange of segments between the X and Y chromosomes during spermatogenesis.

Chapter 11

Down's syndrome and parental irradiation (see p. 233)

Alberman *et al.* (1972) found that the total dose of irradiation received by a group of parents of patients with Down's syndrome was greater than that received by a control group (matched for time of birth and maternal age). The mothers of cases had received significantly more irradiation in the period of their life more than ten years before the birth of the affected child, but this could not be shown for the fathers.

Down's syndrome due to maternal mosaicism

Timson *et al.* (1971) have accumulated data on eight 46,XX/47,XX, G mothers ascertained through the birth of a child with Down's syndrome. The empirical risk of recurrence was estimated at 3 in 5. The average age of the mothers was 23.6 years, and the average percentage of trisomic cells in peripheral blood cultures was 16.4% (range 5–29%). This range overlaps with that found in clinically affected Down's syndrome mosaics. The findings indicate the strong desirability of antenatal diagnosis in such mosaics.

Chromosomes involved in D/G translocations

Hecht and Kimberling (1971) have summarized evidence from autoradiographic and fluorescent studies, and found that of 44 reported DqDq translocations, 34 were 13q14q; and of 76 reported DqGq translocations, 65 were 14qGq. The cause of these distinctly non-random findings is not yet clear, but they serve to explain why offspring with trisomy-13 are seldom if ever born to parents with balanced D/G translocations, whereas children with trisomy-21 are frequently born.

The "cat-eye" syndrome

Evidence is accumulating that the "cat-eye" syndrome, first described in 1878, and comprising *inter alia* colobomata of the iris, preauricular fistulae, and anal atresia, may be associated fairly constantly with the presence of an additional (presumably deleted) chromosome about half the size of a G group chromosome and often satellited. Fraccaro (1971) has described seven such cases, and Darby and Hughes (1971) another case, which also showed abnormal dermatoglyphics (large *atd* angle and high frequency of whorls on the fingertips).

Chapter 13

Agents causing chromosomal breakage

Amongst numerous reports, the following seem worth reporting.

Emerit and Emerit (1972) have confirmed earlier findings by Matsaniotis *et al.* (1966) of chromatid gaps and breaks in direct preparations of bone-marrow

cells from a patient with viral hepatitis. Blood cultures gave cells with "derived chromosome-type changes" (p. 282). This gives strong confirmation that this virus can cause chromatid breakage *in vivo*.

Stevenson *et al.* (1971) have showed that patients treated for rheumatoid arthritis with radioactive gold (^{198}Au) have a high proportion of cells containing damaged chromosomes (8.5%, including 6.3% of cells with dicentric chromosomes). The level is comparable to that in patients who have received spinal irradiation of 250 rads (skin dose) for ankylosing spondylitis.

Badr *et al.* (1971) have found more chromosomal breakage in cells of women taking regular oral contraceptives, or contraceptives by 3-monthly injection, than in controls. The number of breaks in women having contraceptives by 6-monthly injection was significantly lower than in those receiving them more often.

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Addendum B

Addendum to Chapter 15 (J. H. Renwick)

As predicted in the text, the situation is rapidly changing. At the end of 1972, the following assignments have been made, mainly by somatic-cell genetics groups—notably those of Ruddle, Shows, Bootsma, Frézal and Nabholz:

Map-1 (refs A1, A4, A6, A7, A9, A11, A13, A14, A17, A18)	Rh linkage group (Table 12) and Fy linkage group (Fig. 59) augmented by:	
	Aod	Auriculo-osteodysplasia (refs A6, A7)
	AK-F	Adenylate kinase (in fibroblasts: ref. A9)
Map-6 (ref. A12)	MOD (PMDH)	Malate dehydrogenase (soluble, decarboxylating)
	IPO-T	Indophenoloxidase (tetrameric)
Map-7 (refs A12, A15)	MPI	Mannose-phosphate isomerase (ref. A12)
	PK	Pyruvate kinase (ref. A 15)
Map-10 (ref. A8)	GOT-1	Glutamate-oxaloacetate transaminase (cytoplasmic)
	PeD	Peptidase-D
Map-11 (ref. A16)	GPT-C	Glutamate-pyruvate transaminase-C
	KA, Es-A4, LDH-A	As in Fig. 59
Map-12 (refs A16, A5)	GPT-B	Glutamate-pyruvate transaminase-B (ref. A16)
	SHM	Serine hydroxymethylase (ref. A5)
	PeB, LDH-B	As in Fig. 59
Map-13q (ref. A10)	Rma	Retinoblastoma
Map 14 (ref. A12)	NP	Nucleoside phosphorylase
Map-15q (refs A19, A3)	ABO linkage group	(Table 12)
Map-16 (ref. A12)	APRT	Adenine phosphoribosyl-transferase
	Hp_{α}	As in Fig. 59
Map-17q	TK	As in Fig. 59
Map-18 (refs A12, A2)	PeA	Peptidase-A (ref. A12)
	γA18	Immunoglobulin IgA (of sorts: ref. A2)
Map-19 (ref. A12)	GPI	Glucose-phosphate isomerase
Map-20 (ref. A12)	ADA	Adenosine deaminase
Map-21 (ref. A12)	IPO-D	Indophenoloxidase (dimeric)
	AVP	Anti-viral protein

Most of these loci are not polymorphic at a high level. The PGM_1 and 6PGD loci on chromosome 1 are exceptions and have allowed a whole linkage group associated with them to be assigned to chromosome 1. There is still uncertainty about the ordering of the dozen loci now known on that chromosome.

The GPT-C locus, the product of which is scorable in fibroblasts, may or may not be the same locus as is scorable and polymorphic in erythrocytes. If GPT-C is indeed polymorphic it may be possible to map its distance from the LDH-A locus on chromosome 11.

The locus APRT may also be polymorphic, though the temperature sensitive phenotype appears more difficult to score than was implied in the original publication.[10]

Aneuploid phenotypes have at last allowed an assignment. At least provisionally, a retinoblastoma locus is assigned to chromosome 13 and a locus concerned with IgA immunoglobulin to chromosome 18.

The HL-A linkage group is now augmented by the MLC (mixed lymphocyte culture) locus[43] but is not yet reliably assigned, despite several claims.

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