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HUMAN AFFLICTIONS AND CHROMOSOMAL ABERRATIONS

R. TURPIN AND

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HUMAN AFFLICTIONS AND CHROMOSOMAL ABERRATIONS

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HUMAN AFFLICTIONS AND CHROMOSOMAL ABERRATIONS

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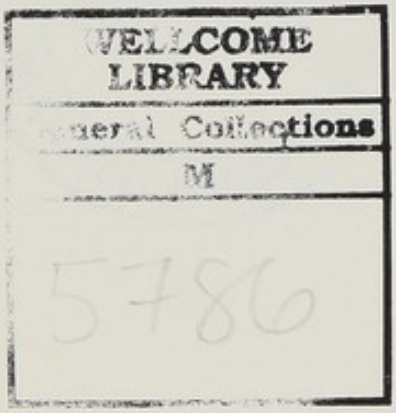

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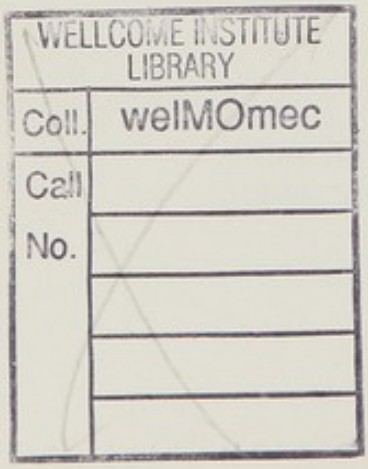

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Contents

Preface to the English Edition

AMONG the great scientific movements initiating the study of human heredity, history will no doubt pick out after Mendelism and genochemistry, chromosome analysis. These fundamental principles have found in human physiopathology a new demonstration of their possibilities since the phenotypic wealth of man, whether healthy or sick, is unrivalled.

This book, *Human Afflictions and Chromosomal Aberrations*, is the English version of the French original of 1965. Only the chapter "Monozygotic Twinning and Chromosome Aberrations (Heterokaryotic Monozygotism)" has been enriched by new advances which increase the interest of the problems raised by this new type of pathological twinning.

As an introductory work it outlines the methods of analysis which lead to the definition of the chromosome apparatus, the karyotype of the normal human somatic cell. The description of these chromosomes, a source through DNA of hereditary information and evolution, introduces the study of their aberrations due to excess or deficiency.

Those who have not participated in this work or have not closely followed it will see that the main models, archetypes and numerical or structural aberrations were isolated in 1959 and were soon confirmed. Their discovery was not the result of mere chance. In fact, the ingenious technique which permits the exact count of the chromosomes of the somatic cell of man, $2n = 46$, did not immediately lead to the discovery of their aberrations. Before these were found it was necessary to find patients likely to bear them. The authors of the discovery of trisomy in mongolism with refined technique at the outset studied this disease to verify a hypothesis advanced 22 years earlier by one of them. The same sequence of events very soon followed in England, where the choice for study of the forms of gonadal dysgenesis was governed by anomalies of the chromatin body.

The discovery of chromosomal aberrations is thus a striking example of the possibilities of confrontation of logic and experience.

These anomalies in our hereditary make-up do not merely have a scientific appeal. They concern all who do not view without anxiety the increase in congenital diseases. A doubtless approximate but reasonable estimate suggests that patent and latent chromosomal aberrations affect some 4 in every 100 zygotes and, after the play of intra-uterine selection, 1 per 100 viable neonates. However, medical and socio-economic progress is more and more reducing the effects of natural selection. Such progress is increasing the life expectancy of these abnormal zygotes and, hence, the importance of the problems they pose.

Some of these problems concern the normal karyotype. A better discrimination of the chromosomes founded on their morphological, biophysical and bio-

chemical attributes is highly desirable. It is essential for the definition of morbid types, the identification of rudimentary structural anomalies, the gene topology of the chromosomes and analysis of a possible relation between chromosome selection and cell specificity.

Other problems concern aetiological factors. It is important to establish the real incidence and make clearer the pathogenetic possibilities: segregation, "replication" and structural anomalies. Certain factors are exogenous: physico-chemical; viral with chromosome tropism. Others are endogenous: inter-chromosomal effects; predisposition of the genes to aberrations; and predisposition to aberrations and cancer.

The first congenital chromosomal aberrations compatible with life heralded a new chapter in physiopathology. They encouraged the hope that progress in the study of human cytogenetics would match that of drosophilian cytogenetics. Now, barely 10 years after the start of these studies, the material for a new chapter in human pathology has been marshalled and continues to increase. Although the progress of this young discipline has not yet fulfilled all the hopes pinned on it, it nevertheless continues to gain in importance and interest.

Paris, July 1968 RAYMOND TURPIN

Preface

THE facts brought to light over the last 5 years in the study of human chromosomes and their pathological variations form the basis of this book. These findings have opened up a hitherto closed chapter in human pathology and have encouraged the development of a new branch of medicine. Though much has been uncovered the knowledge gained will no doubt soon be outmoded. Modern methods afford still unexplored possibilities and refined analytical techniques may perhaps provide a fresh impetus to a study on the point of slowing down. Despite these discouraging aspects and the realization that this book would be far from perfect and contain observations of very uneven merit, we agreed to write it as requested.

We felt it useful to bring to the attention of doctors known examples of congenital pathology which may affect any function or system and be of interest to the widest range of specialists. It also seemed to us of value to compile for the benefit of researchers a bibliography as exhaustive as possible including material published over a 5-year period from the first communication in 1959 to 31 December 1963. Obviously, the bibliography for 1964 cannot be complete. From earlier publications we have taken only fundamental work or facts which have become topical again because of chromosomal discoveries.

Following the Introduction we have divided the book into three parts.

The first deals with the necessary preliminaries, background and definitions followed by the laboratory techniques; then the techniques of study and description of the normal karyotype.

The second is devoted to anomalies in number or structure of the autosomes, in particular, trisomies, translocations and aneuploidies.

The third combines numerical and structural anomalies in the gonosomes—hermaphroditism, twin anomalies and the mechanisms and effects of gonosomal aberrations.

In drafting the last two sections we had to rely on original sources, and present the substance of numerous observations. This is bound to make the text more cumbersome, but the available information does not lend itself to outline form.

It is certain that the order we have chosen will be upset in the near future. Without wishing to prophesy, always a dangerous course in biology, we can expect to see developments in acquired chromosomal pathology. This subject may become, as important, if not more so, than that of constitutional pathology.

Care in assembling the most outstanding contributions does not exclude the risk of omissions. Some readers may perhaps regret that work they consider of value has not been included.

We would make it clear that we welcome any remarks, not viewing them as

criticism but as a contribution to a work which could not in the first instance be entirely successful.

If this work paves the way to new research in the field under study, its purpose will have been amply served. In no way does it claim to be a *summa* but rather a *gradus ad observationem*.

Institut de Progénèse,
Paris, September, 1964

RAYMOND TURPIN and JÉRÔME LEJEUNE

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Introduction

THE remarkable development of cytogenetics of lower species encouraged comparable study of human cytogenetics, which was until recently, unfruitful due to inadequate techniques. Only a few years ago man was still ignorant of the exact number of his chromosomes but then a new technique of analysis transformed the situation namely "hypotonic shock".

Dispersion of the chromosomes made possible examination of *in vitro* mitosis and definitely established at 46 the number of chromosomes in our species.

This new technique was put to immediate use by several laboratories, the first investigations being guided by theoretical notions.

"Mongolism", the initial subject of research, was one of the best studied congenital diseases. Regarded as a genetic disorder, not conforming with classical Mendelian laws, its aetiology was the subject of much discussion. In 1959, the hypothesis of its chromosomal origin was verified by the discovery of trisomy 21, thus establishing the validity of theoretical notions going back a quarter of a century.

Moreover, the indications drawn from study of partial colour blindness and the nuclear chromatin body in intersex states suggested anomaly of segregation of the X chromosome. The discovery, after that of trisomy 21, of the Klinefelter XXY syndrome and the Turner X0 syndrome, confirmed the soundness of these hypotheses.

Finally, the first translocation (G ~ D) was found because the child who carried it displayed a syndrome of widely disseminated physical and mental disorders. This simultaneous involvement of many systems suggested a constitutional anomaly due to a possible chromosomal aberration.

These initial successes, this sudden awareness of a morbid group with far-reaching implications encouraged the setting up of numerous cytogenetic study centres throughout the world, soon followed by accumulation of observations based on more or less firm grounds. And this was only to be expected. But the possibilities of such chromosomal prospects are, in fact, limited by its techniques and its subject.

A. Cytogenetic techniques use only very small samples of material taken from few regions, usually, bone marrow, fascia or skin and blood. Even if more samples, cultures and karyotypes can be obtained, such exploration is marred by two weak points. First, it only extends to certain somatic cells and leaves aside information which might be provided by the germinal cells. Secondly, it always leaves open the possibility of mosaicism.

Cytogenetic techniques permit detection of numerical chromosome anomalies and major structural anomalies. But to ask more would be unrealistic, or even impossible. Within a group, in particular, group 6–12, identification of indi-

vidual pairs is at the verge of feasibility. For the X chromosome the incorporation of ^{3}H-thymidine provides a means of identification whether it be normal or modified.

For certain acrocentrics the value of the satellites as a means of identification is reduced by their variability and even by the possibility of secondary satellization compensating for the loss of one of them, by centric fusion, for example.

There seems to be no point in hoping that present techniques will ever permit identification of fine structural aberrations such as slight deletions or duplications and, even less, inversions.

Giant human chromosomes, whether unrecognized or induced, are still to be discovered.

In these conditions it is not surprising that chromosome topology based on the study of erythrocyte phenotypes and enzymatic reactions is still very rudimentary.

B. The subject of cytogenetics is distinguished by a special diversity in frequency and semeiology.

1. The diversity in frequency, as can be judged from the neonatal period, is certainly quite different from the original diversity.

If a meiotic anomaly is involved, the abnormal gametes are selected for viability and fertility; if a mitotic anomaly is responsible, the pathological ovum has a greater or lesser chance of surviving, depending on the aberration it bears. If this selection did not operate, we should observe varieties such as haplo 21 and 0Y which have not been reported. We should also find a greater number of known varieties, such as the X0 for example.

The first results of chromosome analysis of aborted eggs do not contradict these suggestions.

Some reveal known varieties, others, aberrations incompatible with life, such as triploidies. According to some exceptional observations, the latter appear to be viable only in the mosaic state.

This antenatal selection continues after birth. Thus, the original diversity may be accepted as very probable, since the chances of aneuploidy vary, in principle, with the chromosomes considered and are modified by ante- and postnatal selection. This selection perhaps accentuates the difference in frequency of trisomy 21 and trisomies 13 or 18.

Despite the rigours of selection the varieties of chromosome aberrations are many. It would not be a difficult task to describe them if many of these essential aberrations did not involve different phenotypes.

This leads to increasing complexity for which mosaicism is chiefly responsible. A classic X0 or XXY anomaly may be modified by the joint presence of an XX or another cell line. Depending on the size of the territory occupied by each of these lines and the functional attributes of these territories and the predominant form at the level of the primary gonads, more or less appreciable phenotypic differences can be expected.

Structural aberrations add to this diversity, particularly in relation to gonosomal aberrations. Many involve the X0 anomaly but with nuances which

still require individualization although this is often based on a very small number of observations. It is impossible to pre-judge; only the future will decide. At the moment, we must work within a framework not lending itself to brilliant syntheses.

Other aberrations are concerned with the autosomes. For example, the phenotype of a variety of 22 ~ 13 translocation should theoretically vary with the gene composition of the resulting deletion. According to the known facts this indeed appears to be the case. With time, it is possible that this diversity will be reduced to a few types each related to a particular region of chromosome fragility.

Finally, karyotypic coincidence of aberrations in the number of autosomes and gonosomes, or aberrations in number and structure is possible. Often, one of the aberrations clinically gains the upper hand over the other; aneuploidy, for example, is expressed in its usual form, and translocation often sufficiently balanced to have been inherited is reflected in no sign, at least outwardly. This reservation must be made. However it is quite possible, as well as desirable, that semeiological progress will reveal certain phenotypic characters of translocation hitherto latent.

2. The semeiology of diseases with chromosome aberrations is very characteristic. This feature brings closer together the different forms.

Most of the congenital disorders which make up this new morbid group are characterized by delay in mental awakening and physical development with hypotrophy and disharmony of growth. Against this background appear, depending on the chromosome varieties, visceral malformations, in particular, neurosensory, cardio-aortal, renal and gonadal. These common features have given rise to the idea of a partially non-specific semeiology of chromosome aberrations. This concept is, if nothing else, premature. How can one predict the future of an infant—the type of oligophrenic which he will become? If trisomy 21 is involved, long experience of this disease provides an answer, but for trisomy 13 or 18 or a partial deletion of the short arm of 5, this experience is still lacking. We do not know the psychic future of these abnormal subjects whose life expectancy is very short. It is even possible that their frailty will never allow us to examine them up to the end of their growth period.

As the number of observations increases, anatomical inspection of malformations suggests certain relations between the chromosome varieties and the lesions.

It is not surprising that these early and diffuse aberrations which affect embryogenesis right from its initial stages, have in common the property of disturbing overall physical and mental development of the individual and partial development of some of his mechanisms. But the specificity of the aberrations is reflected in the modes of dysembryogenesis.

Among such modes we would emphasize the importance of symptoms suggestive of a chromosome aberration and hence calling for inspection of the karyotype.

The most typical example is that of the Barr interphase chromatin body, an expression of the inactivation of an X chromosome. Any discrepancy between

the result of chromatin analysis and the sex phenotype points to a gonosomal anomaly.

The lobulation count of the nuclei of granulocytes (nuclear segmentation index or NSI) is less in subjects with trisomy 21 than in normal subjects. This sign is more likely to receive attention now that it is known that the frequency of acute leukaemia is twenty times greater in subjects with trisomy 21 than in normal persons. This increased frequency has also been found for other chromosome aberrations.

Palmar and plantar prints, the relevance of which was until recently limited to trisomy 21, are being more and more explored in the hope of drawing from them a new semeiology.

As for biochemical study, it is mainly represented by a few attempts undertaken not unsuccessfully in subjects with trisomy 21: tryptophan metabolism and enzyme activities (alkaline phosphatase and uridyltransferase in galactose metabolism).

C. The aetiology of chromosome aberrations is just as indeterminate as it was when their study was first initiated.

1. Environmental factors do not appear to be negligible since the overall frequency of trisomy 21 increases with maternal age as perhaps is true of other aberrations (XXY).

From the seventh month of intrauterine life until the start of ovulation oogenesis is quiescent. Many cytologists hold that the primordial oocyte on reaching the end of prophase I enters the dictyotene, and then begins to develop again only with the menstrual cycles.

It was quite natural to relate this physiological feature to the influence of maternal age. The longer the waiting period, the longer the oocyte is exposed to environmental factors. Among them ionizing radiations have been incriminated although the necessary proof has not yet been furnished. Nevertheless, in light of experimental evidence their involvement is very probable.

2. Hereditary factors are also invoked by analogy with experimental work. That humans may be predisposed to the anomalies of chromosome segregation has not been established. But there is good reason to think that factors of predisposition exist.

For a long time, certain hereditary "minor" dystrophies such as epicanthus, furrowed tongue and the transverse palmar crease common in subjects with trisomy 21 have been noted among the ancestors and relatives of these patients with a frequency higher than average. These signs appear more often in subjects with trisomy 21 when their ancestors themselves carried them. There is no evidence to suggest that they are the phenotypic expression of factors predisposing to chromosome aberration. Nor is there any evidence to justify ruling out this hypothesis.

A genic influence is suggested by different chromosome aberrations within families. As this important chapter of human pathology unfolds examples of these coincidences will certainly multiply.

3. When trisomy 21 was discovered, absence in vertebrates of such an anom-

aly led some to liken it to an experimental equivalent in *Drosophila*, the triplo IV fruit fly. Thus, its probable meiotic origin through a disturbance in gametogenesis was suggested. This mechanism of pathological chromosome variation is still valid but it does not exclude other possibilities. The most interesting is an anomaly of chromosome segregation at the time of the first zygotic cleavages which had to be invoked in order to explain mosaicism. The discovery of heterokaryotic monozygotism, consisting of two twins with different karyotypes, XY and X0, therefore a true mosaicism in two individuals, has made the possible mitotic origin of a chromosomal aberration unchallengeable.

D. This study of the relations between chromosome aberrations and physical and mental congenital anomalies was extended to nucleo-cytoplasmic diseases, in particular, leukaemia and cancer. Trisomy 21 here again stimulated this study by virtue of the granulocytes found in it, the NSI of which is particularly reduced, and because of the risk of acute leukaemia which it entails.

This study is well under way. It has already shown that the chromosome apparatus in malignant processes is not necessarily anarchic but may, on the occasion of a leukaemic flare-up, follow an ordered numerical progression. It has shown that certain anomalies are peculiar to certain states, such as the Ph^1 chromosome in chronic granulocytic leukaemia. It seems that the frequency of the Ph^1 chromosome increases during crises and regresses during remissions.

To show the possibilities of human cytogenetic research and its difficulties is not to underestimate the results obtained. The first discoveries have given a new lease of life to human genetics. They pointed to the possibility of reducing the considerable gap which separates the latter from experimental counterparts. Many research teams were then set up. In this way considerable documentation has been built up in 5 years from which the incidence of chromosome aberrations among children born alive or dead may be put at about 1 per cent. The book which we present here is based on numerous published observations. The still insufficient time lapse would not have enabled us, without artifice, to have acted otherwise. We preferred to construct an edifice, the foundations of which are too wide for the still incomplete superstructure.

CHAPTER 1

History

THE development of human chromosome investigations preceded in time the rediscovery of the laws of Mendel since as far back as 1891 von Hanseman reported having counted 18, 24 and more than 40 chromosomes in three cells of normal human tissue.

However, despite the interest shown by cytologists already keenly aware of the fundamental importance of chromosomes, the first observations gave rise to highly conflicting estimates. Thus, Flemming (1898) and Duesberg (1906) proposed $2n = 24$, whereas Moore and Arnald (1906) put the figure at $2n = 32$ and Wilcox (1900) at $2n = 36$. Von Bardeleden (1898) counted 16 and Guyer (1909) 22 chromosomes, and finally, in 1910, Branca in his précis of histology, proposed $2n = 24$ as the somatic norm.

This divergence of opinion was due much more to the poor quality of the preparations than to the actual difficulties of observation.

In fact, all these workers used sections of human testicles. Since most of the samples were taken post-mortem from executed criminals, the tissues were badly damaged even before fixing. Moreover, counting in the sections was made difficult by superimposing of images and also by the possible loss of one or more chromosomes.

The great merit of de Winiwarter was that he selected material from testicular biopsies during surgery, immediately fixing them and obtaining sections of a thickness which made it possible to keep intact the whole chromosome set of one cell (5–7·5 μ). In his historical paper of 1912, de Winiwarter outlined the result of his work which may be summarized in Table 1.1.

When one realizes that the study of thick histological sections makes it necessary to explore the preparation in several focal planes and then to recom-

TABLE 1.1

Equational and reductional mitoses as observed by de Winiwarter (from his paper of 1912)

Cell stage	Chromosome number						
	23	24	25	46	47	48	49
Spermatogonia				2	29	0	1
Spermatocytes I	1	57	2				
Spermatocytes II	10	15	0				
	11	72	2	2	29	0	1
	Total: 117						

bine in one plane the schemes thus obtained in space, one readily appreciates de Winiwarter's *cri du cœur* "I have lost an enormous amount of time in repeating fatiguing and, I also confess, very irksome counts. But I have come across a number of new facts which I consider of importance" (p. 98), and of concluding from his demonstration of the chromosome number that $2n = 47$ in the human male and $2n = 48$ in the female:

> "The abundance of very fine structural details which are usually detected only in suitable material also showed that I was placed in exceptional conditions to succeed—I conclude from this that my results while differing from those of my predecessors have nevertheless a better chance of being the expression of the real situation" (p. 149).

In conclusion, de Winiwarter arrived at the following description "there exist 23 autosomes + 1 heterosome (chromosome X) in the spermatozoids, the diploid formulae being respectively: 46A + X in the male and 46A + 2X in the female".

It is remarkable that despite the enormous difficulties of this type of investigation de Winiwarter arrived at an "expression so close to reality".

Nine years later, Painter (1921) discovered the Y chromosome, a very small unpaired element recognizable as an individual unit at meiosis. In his preparations he found 45 to 48 chromosomes and remarked that in the clearest equatorial plaques so far studied only 46 chromosomes had been found.

However, in 1923 Painter concluded that there exist 48 autosomes including 23 autosomal pairs and two unpaired sex chromosomes. The chromosome formulae then became: 46A + X + Y for the male, and 46A + X + X for the female.

Since the figure of 48 corroborated de Winiwarter's observations (with addition of Y) these conclusions were generally accepted and the number 48 was regularly reported by later workers: Evans and Swezy (1929), Kemp (1929), Andres and Vogel (1936), King and Beams (1936), Koller (1937), Léo Sachs (1954), and so forth, and all the manuals on human genetics took it for granted up to 1956.

However, highly important technical progress was made by Kemp who as early as 1929 used tissue cultures to observe somatic mitoses, while Krutschov and Berlin in 1934 observed mitoses in cultures of white blood cells. However, for these new methods to be applied it was necessary to await a new technique introduced by Hsu (1952). He observed that hypotonic shock shortly preceding fixation permitted dispersion of the chromosomes and allowed easier identification of individual chromosomes.

It was in 1956 that Tjio and Levan using tissue culture and hypotonic shock discovered that our species has only 46 chromosomes.

We may wonder in retrospect why these two extra chromosomes were accepted for such a long time. Perhaps this was a result of technical imperfections but

TABLE 1.2

Number of mitoses observed by Tjio and Levan in 1956 (human embryo lung culture)

45	46	47 and 48	
0	261	4	Total: 265

if we apply the observations of Koller (1937) of very early disjunction of the X and Y chromosomes to the findings of de Winiwarter, the observations of the latter agree with the figure of 46. Likewise, the initial observations of Painter were, on his own admission, closer to 46 than 48.

All subsequent work seems to have been influenced by the desire to bring the conclusions of the authors into line with previous publications. This sacrifice to the principle of authority allowed an error to prevail for almost 30 years. A similar phenomenon was also noted by Tjio and Levan (1956) commenting on the observations of Hansen–Melander, Melander and Kullander. These workers, studying mitoses in human embryo liver cells and regularly counting only 46 chromosomes, temporarily abandoned their investigations "because these researchers did not succeed in finding 48 human chromosomes in their material".

However, accurate though it was, the correction by Tjio and Levan only applied to somatic tissue of embryos in medically induced abortions. The determination of the chromosome number in the sex cells was made by Ford and Hamerton in 1956 a few months later. Abandoning the old method of embedding in paraffin and tissue sections, these authors teased out freshly removed tubules and thus obtained directly observable isolated cells. This method avoids "loss" of chromosomes when the razor unfortunately separates certain elements from the remainder of mitosis and, moreover, permits use of hypotonic shock.

The results of Ford and Hamerton finally confirming the figure of 46 are presented in Table 1.3.

TABLE 1.3

Number of masses present in spermatocytes I from diplotene to the metaphase (after Ford and Hamerton, 1956)

Patient	Age	22 bivalents or less	23 bivalents	22 bivalents + X + Y
No. 1	63 years	9	81	11
No. 2	53 years	3	39	11
No. 3	47 years	2	29	3
		14	149	25

Finally, it is important to stress that the new studies of Hamerton (1961a) fully confirmed previous reports and that so far no other observations of extra chromosomes in the sense of Kodani's "supernumerary" (1958) have been reported.

Although it is urgent to resume on a much larger scale studies of meiosis in man, it seems legitimate to conclude that the figure of 46 is definitely established even for seminal cells. No recent document concerning oogenesis is now available.

As regards the somatic cells the evidence accumulated in different laboratories is impressive. At the start of 1960 the results of examination of over one hundred normal individuals had been published by different workers, analysis of 4246 mi-

toses revealing a normal set of 46 chromosomes (cf. Lejeune, 1960a). At the start of 1963, these figures had to be multiplied by ten to correspond with the accumulation of evidence.

The constancy of the normal human karyotype is remarkable and the relative ease of identification of each of its elements soon enabled each laboratory to establish as early as 1958 and 1959 its own classification of human chromosomes. In June 1960 a working group meeting in Denver compared the various classifications of Ford, Jacobs and Lajtha (1958), Tjio and Puck (1958), Chu and Giles (1959), Lejeune, Turpin and Gautier (1959), Böök, Fraccaro and Lindsten (1959), and Levan and Hsu (1959); arriving at an international classification now universally employed. Since then, a new classification deriving in the main from those of Ford *et al.* and Lejeune *et al.*, has been proposed by Patau *et al.* (1960b).

As we shall see later, the international classification is in no way perfect but represents a simple means for transmission of information between specialists and it seems legitimate to recommend its use in all publications to the exclusion of any other.

This rapid outline of recent developments in chromosome studies does not take into account the pathological point of view which in the final analysis is the only *raison d'être* of the present work.

To sum up briefly, we may say that systematic study of the human karyotype began in 1956 with the publication of Tjio and Levan while study of chromosome aberrations came 3 years later in 1959 with the discovery of trisomy 21 in mongolism (Lejeune, Gautier and Turpin, 1959e) and immediately afterwards, with the discovery of the XXY sex-determining mechanism of Klinefelter's syndrome (Jacobs and Strong, 1959). The development of chromosome pathology was spectacular, in less than a year the four most frequent syndromes were discovered: trisomy 21, XXY formula in the Klinefelter syndrome, X haploidy in the Turner syndrome (Ford, Jones, Polani, de Almeida and Briggs) and the triplo-X syndrome (Jacobs, Baikie, Court Brown, MacGregor, Maclean and Harnden). To these syndromes must be added the first example of translocation observed in man (Turpin *et al.*, 1959a) published 5 months after the discovery of the chromosomal anomaly of mongolism.

The years 1960 and 1961 were also very eventful with isolation of the trisomy 13 (Patau *et al.*) and 18 (Edwards *et al.*) syndromes, all the constellations of sex chromosomes ranging from XY to XXXXY and from X to XXXX and numerous examples of translocations, deletions, etc.

Although it is not absolutely certain, it seems that now the pioneering period is over in the sense that the very frequent malformation syndromes compatible with life resulting from major chromosome aberrations (affecting over 1 or 2 μ of chromosome material), have already been observed. This does not mean in any way that the whole field of human chromosome pathology is already known but only that the easiest identifications have already been achieved. Nevertheless, multiplication of observations and refinements of technique will alone enable us to detect minor or very rare aberrations and to grapple with the difficult and almost entirely unexplored problem of acquired chromosome pathology.

CHAPTER 2

Techniques of Studying Human Chromosomes

A. CULTURE TECHNIQUES

There are now many techniques for studying human chromosomes, each laboratory having evolved its own variant of different already published methods. A historical review of this subject would be tedious and we shall only try to present to the reader an outline of the various possibilities describing those which by usage have proved to be the simplest and most faithful. This inevitable selection does not imply any unfavourable judgement on other practicable techniques.

In particular, the techniques described below can be applied without controlling the atmosphere of the incubator, which avoids the use of delicate equipment. All the necessary manipulations are bacteriological routine and the only essential material consists in several Pyrex tubes and a heated cabinet correctly stabilized at 37°; this may be replaced by a simple, controlled temperature water bath.

We deliberately insist on this point: although chromosome studies require much care and application they call for only minimal outlay on materials (apart from the use of a good microscope permitting photomicrography and can be undertaken in any biology laboratory.

Chromosome examinations have to be made by many investigators and it can be said that within a few years no large hospital or medical school will be able to manage without at least a cytogenetics laboratory.

It is to facilitate these essential developments that the present chapter was written so that study of the karyotype now considered an exceptional form of investigation becomes a diagnostic examination applicable to all cases of constitutional anomaly. Moreover, examination of the chromosome set of mutant clones, neoplasms for example, is bound to develop rapidly and perhaps one day it will even exceed in importance study of constitutional anomalies.

General remarks

Since the aim of cytological investigations is to establish the karyotype of the cells examined, all techniques whatever their working principles and whatever the origin of the tissue must satisfy three demands:

(1) dividing cells must be obtained showing adequate spreading of the chromosomes (in order to count them);

(2) treatment must entail minimal deformation (in order to identify them); and

(3) the preparations must be flattened (in order to obtain photographs in which all the chromosomes are simultaneously in focus).

Obtaining dividing cells

The first idea of observers was to resort to tissues undergoing natural proliferation and this is one of the reasons why the first counts were made on testicular tissue (Chapter 1). More recently tissue cultures have been used and, finally, cultures of the haematopoietic system—bone marrow and circulating blood.

Whatever the method of culture two possibilities exist for stepping up mitoses: either obtain a "wave" of mitoses by controlling the nutrient medium, which makes it possible to observe normal mitoses; or block the anaphase in order to accumulate metaphase figures by using the mitostatic action of colchicine and its derivatives, discovered by Gavaudan, Gavaudan and Pomriaskinsky-Kobozieff in 1937.

When mitoses are relatively rare (short-term blood and bone marrow cultures) colchicine is almost indispensable. On the other hand, when cell growth is intense (tissue cultures proper) it becomes possible to study the early stages, prometaphases, for example, and colchicine is then superfluous. In fact, the mitostatic action of colchicine is not restricted to mere inhibition of the spindle but produces an anomaly of the chromosomes themselves as amply shown by Amarose (1959) and disturbance in the rate of DNA synthesis (Lima de Faria and Bose, 1962). Finally, pre-anaphase separation of centromeres and even sometimes chromosomal fragmentation at the centromeric level makes it even more difficult to interpret the images. We therefore consider that the use of colchicine must be avoided in all cases where the technique does not require it.

Spreading of chromosomes

The jumble of chromosomes like the pieces in spillikins for long remained one of the major obstacles to counting them. Their dispersion by hypotonic shock as discovered by Hsu (1952) solved this problem, the shock scatters the chromosomes in the swollen nucleus, just like the tap on the table scatters the pieces in the game of spillikins. Despite its unquestionable usefulness this dispersion means that some of the desirable information is lost, especially with regard to the probable association of chromosomes with satellites between them, the possible somatic pairing of homologues or even a spatial structure characteristic of the equatorial plate.

These two latter possibilities were recently studied by Barton and David (1963) and Barton *et al.* (1963a, b) who concluded that there is a tendency towards association between homologous chromosomes especially in female cells. O. J. Miller *et al.* (1963a, b) consider that certain chromosomes tend to be found

more frequently than others at the periphery of mitotic figures, following dispersion of the chromosomes. No simple explanation of this phenomenon has yet been proposed.

Moreover, hypotonic shock must be moderated in order to avoid disturbing the very structure of the chromosomes.

Treatment of preparations

All methods of dispersion, fixation and staining now used are effective but not equally so. The squash method or crushing technique has certain advantages over hypotonic shock (chemical integrity of the chromosomes) but serious drawbacks: mechanical deformations especially loss of chromosomes.

Difficulties in fixing and staining may be resolved in one stroke by the use of aceto orcein (cf. p. 16) but the preparations are unstable, although a technique of Chu and Giles (1959) makes possible permanent specimens. Moreover, the contrast between the chromosomes and the nuclear fluid leaves much to be desired and identification of individual satellites (Chapter 3) is often insufficient.

For these reasons we consider it essential to obtain permanent preparations from the outset with differentiation of the chromosomes by prior hydrolysis of the cytoplasm.

Finally, flattening of the preparation by simple air drying (Rothfels and Siminovitch, 1958) is fully satisfactory and a common feature of most techniques.

Special techniques

As can be readily appreciated, the techniques of chromosome study must be modified in relation to the tissue employed. We shall therefore present the techniques for testicular tissue, bone marrow, peripheral blood, and, finally, tissue biopsies of any origin.

1. Study of meiosis

In the female, study of meiosis is made particularly difficult by the fact that all the oocytes have completely finished the first meiotic prophase several weeks before the birth of the individual.

After this preparation for segregation of the two haploid sets, the oocyte remains in a stage known as the dictyotene up to fertilization. Finally, at the moment of ripening of the follicle, after rapid condensation of the chromosomes, the first polar body is expelled, involving one of the haploid sets. Expulsion of the second polar body (equational mitosis) occurs only after ovulation and fertilization. The diploid mitoses of the oogonia and the leptotene, zygotene, pachytene and diplotene stages of the reductional prophase were recently studied by Ohno, Klinger and Atkin (1962c) in ovaries of human 3–9-month-old foetuses.

The difficulty of ovarian biopsy and also the need to include in the specimen the ripening follicle have so far prevented study of reductional meiosis in the female. Likewise, equational meiosis, which is contemporary with fertilization occurs too late and thus cannot be studied in our species.

In the male, on the other hand, the continued activity of spermatogenesis after puberty coupled with the inocuity and the relative ease of biopsies permits examination on fresh material a few moments after sampling.

The technique described below is that of Ford (1961), the details of which were kindly communicated to us by the author. Previous publications of Ford, Kodani and Sachs may be consulted for further information.

Technical directions after C.E. Ford, 1961

(1) Place the fragment in hypotonic solution and tease out tubules with blunt needles or seekers and leave the preparation at room temperature for 3 min. Ten to 20 mm^3 testicular tissue is sufficient for normal individuals.

(2) Transfer the tissue to a 3:1 mixture of absolute ethyl alcohol and glacial acetic acid as fixative. Leave 1 hr.

(3) Hydrate, by replacing the fixative first with 30 per cent alcohol, then with distilled water. Leave in each fluid for 3–5 min.

(4) Hydrolyse by transferring tissue to normal HCl at 60 °C and leaving for 8 min.

(5) Stain with Feulgen reagent 1 hr.

(6) Remove Feulgen reagent by rinsing in SO_2 water, followed by chilled 45 per cent acetic acid, 3–5 min each.

(7) Preparations may now be made or the stained tissue may be stored in 45 per cent acetic acid at -12 or -15 °C for later use.

(8) Preparations are made by transferring three of four short lengths of tubule (1–2 mm) to a clean glass slide separating each of them in a drop of 45 per cent acetic acid; they are covered with a flat siliconed cover-slip and then gently squashed between two or three layers of filter paper. Pressure is applied evenly by both thumbs together, one on each end, and gradually increased, taking care not to impart any sideways motion to the cover glass. The amount of pressure to be applied varies with the specimen and can be judged by making a first rough preparation for each examination.

(9) It is frequently advantageous to treat the tubules with 1 per cent papain solution in water for 10 min or more immediately before squashing. The tubules should be rinsed in water before and after treatment with papain. They shrink considerably in papain but swell to greater than the original size after being returned to 60 per cent acetic acid in which they should remain for about 5 min before being covered and squashed. Treatment with papain greatly improves the spreading of the cells, probably by digestion of the basement membrane of the tubule.

(10) The preparations are made permanent by freezing in carbon dioxide snow. If this is not possible the preparations can be left overnight in 95 per cent alcohol, Cellosolv, dioxane or other dehydrating agent. On the next day the

cover slips are carefully separated with a razor blade, dehydration is completed and the preparations are mounted in Euparal or Canada balsam.

Notes: (a) The hypotonic solution used is made up as follows with pH adjusted to 7·0

potassium glycerophosphate	50 per cent	93·02 ml
glycerophosphoric acid	20 per cent	24·92 ml
water	to	200 ml

(b) To "silicone" the cover-slips simply rub them with a cloth impregnated with silicone of the type used for "demisting" motor car windscreens.

(c) The use of colchicine makes it easier to investigate spermatogonial metaphases but does not appear to have any worthwhile action on the chromosomes of the spermatocytes.

2. Study of bone marrow

(a) Short-term culture technique as proposed by Ford, Jacobs and Lajtha, 1958

(1) The sample of bone marrow (1 ml if possible) is dispersed in Ringer's solution containing 1:20,000 heparin. The cells are spun down by mild centrifugation (500 to 1000 r.p.m.) and the supernatant discarded.

(2) The cells are then resuspended in isotonic glucose–saline mixed in equal parts with human AB serum (or the serum of the subject himself). Aliquots of 2–4 ml are transferred to McCartney bottles. The cultures at this stage are kept at normal temperature overnight, and this time lapse be may used for transport (at ambient temperature).

(3) The tubes are then placed in an incubator at 37° (or on a water bath). The best yield is obtained by incubation for 5 hr followed by addition of 0·2–0·4 ml of 0·04 per cent isotonic saline solution of colchicine. After 2-hr further stay at 37° the medium is diluted four times by adding 0·37 per cent sodium citrate solution. After 10-min stay in this hypotonic solution the cells are recovered by centrifugation and elimination of the supernatant followed by fixation and staining by the aceto orcein technique. A variant of this method is to allow colchicine to act immediately on the marrow sample so reducing the total time between sampling and fixation to 2 or 3 hr (Tjio and Wahng, 1962). This technique is particularly useful in study of leukaemias since it avoids any *in vitro* selection.

(b) Medium-term culture (liquid medium technique)

This method described by Fraccaro, Kaijser and Lindsten (1960) and adopted by Hirschhorn and Cooper (1961) may be summed up as follows:

(1) 1 ml of bone marrow aspirated by sternal or iliac puncture is immediately suspended in 3 ml of culture medium composed of: AB serum 35 per cent, medium 199 60 per cent and embryonic extract 5 per cent.

(2) This mixture is then distributed in Petri dishes containing a coverslip and incubated at 37° for 24–72 hr. The atmosphere of the incubator must be controlled at 5% CO_2.

(3) After 2–3 days the coverslips are withdrawn and transferred to hypotonic medium (0·7 per cent sodium citrate) for 10 min and fixed and stained (orcein).

This technique gives excellent results but requires control of the atmosphere of the incubator and to avoid this drawback we personally use the following modification.

Solid medium technique

(1) Place on a disc in a Leighton tube covered with a film of chick plasma (cf. § 5(c)) one or two drops of bone marrow aspirated in a heparinized syringe.

(2) Coagulate the plasma with one drop embryonic extract.

(3) Add the following nutrient medium: medium 199 5 drops, AB serum (or patient's serum) 5 drops and embryonic extract 2 drops.

(4) Seal hermetically and incubate at 37° for 3–5 days regularly following the growth with a microscope in order to choose the best moment for replenishing the culture.

(5) Replenish, then prepare the discs (dispersion, staining) exactly as under protocol § 5(c).

This technique is in effect an application of our method of tissue culture to study of bone marrow and avoids checks on the atmosphere of the incubator, making it possible to use the same glassware for different samples and gives results quite comparable with the preceding version from which it does not differ in principle. Use of colchicine is optional.

(c) Immediate examination

A variant of the Ford method proposed by Bottura and Farrari in 1960 represents application to man *in vivo* of the use of colchicine already used by Ford in the animal. Bottura and Farrari (1960) proposed intravenous injection of colchicine into the patient 2 hr before bone marrow puncture. The marrow thus obtained is at once treated with hypotonic medium then fixed and stained, colchicine having blocked mitoses in the metaphase *in vivo*.

This method is little used for obvious medical reasons. Injection of colchicine in doses compatible with those employed in attacks of acute gout cannot be considered fully safe. It therefore seems more legitimate to allow the drug to act *in vitro* rather than *in vivo* even if this measure of prudence imposes a more meticulous manipulation on the part of the investigator.

3. Study of peripheral blood

Initiated in 1934 by Krutshov and Berlin, the study of mitoses obtained by peripheral white cell culture was restored to a place of honour by the work of Moorhead *et al.* (1960) and Nowell and Hungerford (1960). It would appear that the cells capable of dividing *in vitro* are of the type of small lymphocytes (Carstairs, 1961) and the experiments of Porter and Cooper (1962) on the rat provide cogent demonstration of this notion (metaphases bearing the Y chromosome after injection of male rat lymphocytes into newborn female rats).

The following protocol makes use of this method with some variation and can be applied a few hours after sampling. A method for preserving total blood at $-80°$ with addition of 15 per cent glycerol before freezing (Atkins, 1962) makes it possible according to this author, to postpone the culture for several months.

(a) Culturing

(1) 20 ml venous blood is withdrawn in a heparinized syringe (1 ml of solution of 5000 I.U. heparin per ml is drawn into the syringe the walls of which are uniformly coated and then the heparin is completely discarded).

(2) The blood is decanted into a test tube inclined at 45° and the red cells allowed to sediment spontaneously. It can also be kept in the syringe the needle held upwards during sedimentation. After a period varying from 30 to 50 min the sedimentation is sufficient to permit the withdrawal of the supernatant which contains at this moment exclusively white cells—5 to 10^6 per ml.

This simple decantation already proposed by Edwards and Young (1961), avoids slow speed centrifugation (200 r.p.m.) the main drawback of which is an occasionally catastrophic loss of white cells.

Moreover, addition of Bacto phytohaemaglutinin (Difco) after decanting ensures that many leukocytes are not trapped in the micro coagulates of red cells.

(3) An aliquot of supernatant liquid containing white cells is then studied in a Malassez counting cell. The aim is to obtain a final concentration of 1 to $1{\cdot}5 \times 10^6$ cells per ml in a medium consisting of 30–35 per cent of the patient's serum and 65–70 per cent of medium 199. To do this, depending on the previous count, the desired proportions of the patient's serum and medium 199 are again added.

0·2 ml of Bacto phytohaemaglutinin is added per 10 ml of mixture which is then distributed in ordinary test tubes (Pyrex) in such a way that the tubes are filled to one-third their volume. It is possible to inject into the remaining space a mixture of air and 5% CO_2 just before sealing the tube. This precaution considered by some as essential does not appear to us of crucial importance.

(4) The tubes, carefully sealed, are then incubated for 72 hr.

(b) Accumulation of mitoses

The number of mitoses observed at the 72nd hr displays considerable variation from one culture to the next. It is advisable to add 2 hr before the manipulation 2 drops of isotonic solution of colchicine (or a synthetic derivative) at a concentration of 0·04 per cent per ml of medium in order to obtain the maximum number of analysable metaphases. This time of action of colchicine can be reduced to 1 hr or even completely omitted (Hirschhorn).

(c) Making the preparations

(1) After decanting the contents of one test tube into a conical centrifuge tube they are spun down at 800 r.p.m. for 5 min in order to sediment the white cells.

(2) The supernatant is discarded and replaced by hypotonic solution. A 0·93 per cent solution of sodium citrate (hypotonic human serum solution, cf. § 5(c), can also be used) is added for 10 min at 37°.

(3) Further centrifugation at 800 r.p.m. helps to remove the hypotonic solution which is replaced by Carnoy's mixture described under § 5c. The fixative must be added by allowing it to run down the side of the tube and by progressively bringing the cells into suspension by tapping the tube. It is left in contact for 35 min.

(4) Further centrifugation at 800 r.p.m. then removes the Carnoy's fluid which is replaced by two to three drops of fixative in which the cells are resuspended by careful pipetting avoiding bubble formation absolutely. Here, the Carnoy's mixture used is 3 vol. of ethyl alcohol and 1 vol. of acetic acid.

(5) Then a sheet of ordinary paper is placed on an ice block or even better on the bottom of an upturned ice-tray. Use of carbon dioxide snow has also been proposed (Hamerton). Several perfectly clean coverslips (washing with soapy water, rinsing and prolonged stay in alcohol) are placed on the paper and by cooling the slides may be expected to be covered with a very fine mist.

On each of them a drop of cell suspension is then placed which immediately spreads over the whole coverslip. It is then held above a flame, or better, placed in the warm air cabinet at 60° to obtain rapid evaporation of the fixative necessary for flattening the preparation.

(6) Then the coverslips are allowed to dry completely in air and can be stored almost indefinitely in this form. Subsequent staining and mounting are described under § 5(c).

The use of aceto orcein described previously may be considered for very rapid preparation without great demands on quality.

4. Microtechniques

Inspired by the micromethods of Edwards (1962) and Frøland (1962) our method currently uses several drops of total peripheral blood. Arakaki and Sparkes (1963) and Grouchy *et al.* (1964c) have described a similar technique.

Because of the simplicity of sampling and culturing, permitting sampling away from the laboratory, we consider the micromethod as the method of choice for study of blood.

Materials

Pyrex round-bottomed centrifuge tubes.
Screw-capped bottles with pure siliconed washers.

These tubes with a useful capacity of about 41 ml are filled in advance with the following medium: 5 ml human serum (serum of group AB or the subject's serum), 15 ml medium 199, 4 drops of phytohaemaglutinin,† and 4 drops of Liquemin (Roche)‡.

This mixture can be prepared in advance and kept on ice.

† Mixture in equal parts of Phyto Difco M and P or Phyto Wellcome.
‡ Or the equivalent of 5 mg crystalline heparin.

Sampling and culturing

(1) Carefully disinfect the skin of the finger (index) or thumb by washing with soap and careful rinsing, press twice on the site for 1 min with pad impregnated with alcohol and dry by rubbing over with pad impregnated with ether.

(2) Incise the skin with a vaccinostyle (as for blood cell counts) and remove 4–6 drops of blood with a Pasteur pipette.

These four drops of blood are directly transferred to the tube containing the medium and the pipette rinsed with the same medium to allow the blood to suspend. It is also possible to use 0·2 ml of blood taken from a vein.

(3) Carefully close the tube and leave it in the incubator at 37° for 48–72 hr.

Swelling and fixation

(1) After a stay of 48–72 hr in the incubator at 37°, add 2 ml of an 0·04 per cent isotonic solution of colchicine.

The appearance of the culture is then rather deceptive and the existence of a true clot should not in any event be taken as a sign of failure. Resuspend all the cell aggregates and leave in the incubator for 2 hr.

(2) After further careful resuspension transfer the contents to a conical centrifuge tube.

(3) Centrifuge for 5 min at 800 r.p.m. and discard the supernatant.

(4) Fill the tube two-thirds full with a mixture of 1 vol. animal serum, 5 vol. distilled water and hyaluronidase 2·5 I.U. per ml of mixture.

Carefully resuspend and leave in the incubator for 7 min.

(5) Again centrifuge at 800 r.p.m. for 5 min in all, allow an interval of 2 min between the different manipulations since the cells must remain in contact with the hypotonic liquid for about 14 min.

(6) Discard the hypotonic medium and add the Carnoy fixative (3 vol. chloroform, 1 vol. glacial acetic acid and 6 vol. absolute ethyl alcohol) while gently resuspending.

A period of 45 min is necessary for complete fixation.

(7) Again spin down at 800 r.p.m. for 5 min; discard the Carnoy's solution and replace by the following fixative: 1 vol. glacial acetic acid to 3 vol. ethyl alcohol.

The hermetically sealed tube can then be kept in a refrigerator for subsequent use.

Spreading and staining

(1) Again centrifuge at 800 r.p.m., discard the supernatant and add 5–6 drops of fixative in which the cells are resuspended by careful pipetting. It is important not to produce air bubbles during any of all these manipulations.

(2) Place very clean slides on an ice block the surface of which must be more or less flat.

(3) Take the slide and drain it by tapping the side against blotting paper in such a way that the surface which has been in contact with the ice shows a very thin but continuous film of water.

(4) Allow a drop of fixative containing cells to fall onto the film of water the tip of the Pasteur pipette being 3 or 4 cm away from the slide.

(5) Allow the drop to disperse spontaneously in the film of water for 30 sec to 1 min.

(6) Leave to dry in the open or in an incubator at 50 or 60°.

(7) After complete drying, hydrolyse for 7·5 min in normal HCl at 60° and rinse in iced water.

(8) Stain for 10 min with Unna's blue according to the technique on p. 23.

(9) Mount in Canada balsam covering the preparation with a wide cover slip.

5. Study of tissues taken at biopsy

In general, any tissue aseptically removed is capable of giving usable cultures. Various techniques have now been proposed essentially differing in the way in which the cells are transferred; either *en bloc*, in the state in which they are in the fragment (explant technique) or after trypsinization (Harnden, 1960).

It is important to note that the method below developed for culturing samples of connective tissue during surgical intervention (fascia, perimuscular aponeurosis) can be applied without any modification to samples of other tissues: serous, subcutaneous, visceral, epithelial or tumor. In the case of skin biopsies which represent the easiest material (after peripheral blood) it is necessary to avoid any local anaesthesia.

The biopsy technique employed by us is the following:

(a) Surgical biopsy

(1) Aseptically remove under general anaesthesia a fragment of connective tissue measuring about $4 \times 4 \times 4$ mm (or any other tissue to be examined).

(2) Place the fragment on a sterile square of gauze and draw in the corners to wrap it.

(3) Place the gauze square in a wide-necked flask of at least 4 cm diameter. It must be possible to seal this sterile flask hermetically (ground stoppered or plastic caped).

(4) Pour the contents of one ampoule of sterile physiological saline (5 ml) onto the gauze in the flask.

(5) Close the flask and transport it at normal temperature (neither in ice nor incubator).

(6) The time lapse may be 24–36 hr and transport over long distances is perfectly feasible.

All manipulations, biopsying and flasking must be made by the surgeon himself with rigorous asepsis.

(b) Skin biopsy in the laboratory

Using the technique previously proposed by Edwards (1960) we observe the following protocol:

(1) Carefully wash a region of glabrous skin with ordinary soap.

(2) Carefully rinse with sterile distilled water.

(3) Cover the region with a pad impregnated with alcohol which is left in position for 1 min.

(4) Change the pad twice and replace it by another impregnated with ether.

(5) After complete spontaneous drying pinch the skin between the flexible teeth of the "Coprostase clamp" type. The clamp is locked after making sure that a skin fold of 2 mm height protrudes above its upper margin. The length of this fold must be 3–4 cm. This clamping is a little disagreeable but quite tolerable if the jaws of the clamp are very flexible and it is the only painful period of the intervention.

(6) Cover the fold held by the clamp with a new pad impregnated with ether and leave it in position for at least 2 min, renewing the ether once. This interval of 2 min is indispensable for anaesthesia by compression.

(7) Then remove with a scalpel the central region of the fold scraping the upper edge of the clamp—in this way one should obtain a fragment 3–4 mm long and 2 mm thick at its centre. The incision must be made at the centre of the fold, the only site really anaesthetized by compression.

(8) The fragment is then placed on a square of gauze and can be manipulated according to the technique described above for surgical biopsy.

(9) Without relaxing the clamp, a little mercurochrome is smeared on the surface of the wound which does not bleed and it is covered with a sterile pad.

(10) After a 3 min wait necessary for spontaneous haemostasis the clamp is withdrawn and the skin brought together with the aid of sticking plaster or band aid so that the margins of the wound approach one another.

(11) The dressing is removed at the end of 6 days and is not replaced; the scar should be extremely discrete.

Note. The site of choice in the child is the infraspinal fossa because of its easy access and its very poor innervation. In the adult, expecially if there is cellulitis, sampling should be done at a site where the skin is very loose, on the medial aspect of the upper arm or the anterior aspect of the forearm.

(c) Method of explants (after Lejeune et al., 1960)

Whatever the method of sampling, culturing and the various manipulations are carried out according to an identical protocol.

The fragment is washed in physiological saline and divided with new scalpels into small explants of about 1 mm to 2 mm on each side.

The culture is carried out in Pyrex tubes with a distal depression in which a coverslip rests† (Fig. 2.1). All the glassware must be of Pyrex and washed very carefully (avoid synthetic detergents), rinsed several times in distilled water and autoclaved.

The media are deposited with Pasteur pipettes used only once and the explants are manipulated with identical pipettes but with a curved tip.

† "Leighton tubes" or tubes for tissue culture of the Pasteur Institute type.

Culturing consists of first spreading a drop of chick plasma† over the coverslip lodged in the tube. Then the explants (2–3 per coverslip) are placed on this plasma film. At this moment the addition of a drop of embryonic extract‡ coagulates the plasma and fixes the explants to the glass.

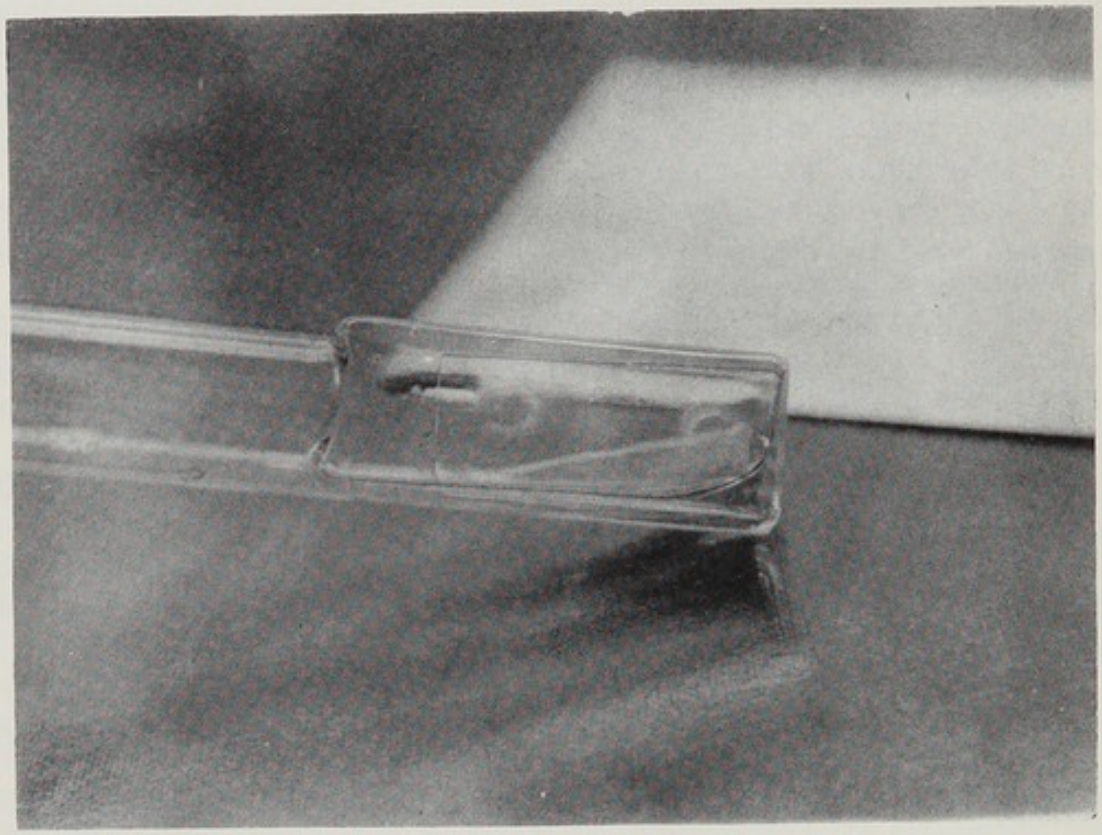


FIG. 2.1. Tube with glass slip. Note the two rows of cells growing directly on the microscope coverslip.

Then the tubes closed with grey non-toxic rubber stoppers are left for several hours, or, if need be, overnight, in an incubator at 37°. After this time lapse the culture medium is added consisting of the following ingredients per tube 5 drops of human serum (preferably of group AB), 5 drops of Hanks's solution containing penicillin 200 U/ml, streptomycin 50 μg/ml, chloramphenicol 5 μg/ml and 2 drops of embryonic extract.

It is always prudent to maintain simultaneously at least three tubes per biopsy enabling one to obtain parallel but independent cell lines. This is important in order to eliminate with certainty the possibility of an *in vitro* chromosome mutation.

† This chick plasma is obtained by puncturing the marginal vein of the wing and aspiration of the blood in a syringe containing 1 ml heparin. The blood is immediately centrifuged at 3000 r.p.m. for 10 min and the plasma stored on ice in a hermetically sealed sterile tube.

‡ The embryonic extract from eggs incubated for 9–10 days is, in fact, the supernatant of the crushed material diluted 1:1 with Hanks's solution.

Transfer

After a period varying from 4 to 6 days, growth of a crown of fibroblasts around the explants is observed. The explants are then removed and transferred to other tubes according to the above technique if one wishes to continue the culture.

The medium contained in the tubes with coverslips is then replaced by fresh medium of identical composition.

Accumulation of mitoses

After a further stay of 36 hr in the incubator, 3 drops of embryonic extract are added to each tube without changing the medium. The tubes are again placed in the incubator for an additional period of 16 hr. At this moment, a very large number of mitoses should be observed, chiefly prometaphases, the most favourable stage.

Dispersion of the chromosomes

With the aid of a Pasteur pipette with a curved tip the coverslips are removed from their tube and then placed cells uppermost, into the following solution at 37° and kept there for 35 min: 1 part sterile human serum, 5 parts pure, neutral water plus hyaluronidase 2·5 I.U. per ml of mixture.

Mammalian serum (foal, for example) may replace the human serum.

Fixation

Once withdrawn from the hypotonic solution the coverslips are placed for 45 min at laboratory temperature in Carnoy's solution: 3 parts chloroform, 1 part acetic acid and 6 parts absolute ethyl alcohol.

Flattening of the preparations

The preparations taken out of Carnoy's are left to dry in the open air.

Staining

(1) *Hydrolysis:* The coverslips are placed cells on top into a beaker containing normal HCl for seven and a half minutes at 60° which is sufficient to eliminate any stainable cytoplasmatic structures. At the end of this period the coverslips are carefully rinsed with pure, neutral water (spring water).

(2) *Staining with Unna's blue.* Immerse the coverslips (always with cells on top) for 10 min in a solution of 1 part of Unna's blue solution† and 4 parts of pure, neutral water.

(3) Withdraw and lightly rinse with neutral water to remove surplus stain.

(4) Leave to dry in open air to complete desiccation (5–10 min). By this means is possible to avoid the standard ethyl alcohol passage which may remove part of the dye fixed on the chromosomes.

Then the preparation is mounted, cells between the slide and the coverslip, in Canada balsam after steeping in toluene to avoid air bubbles.

† Employ R.A.L. Unna's blue solution.

(d) Liquid medium method

The surgical specimen collected aseptically is placed, wrapped in a square of gauze, in a sterile flask containing Hanks's solution or in its absence in isotonic saline.

(1) In the laboratory the specimen is placed in a Petri dish and freed of the fat which may surround it. It is then cut into small fragments measuring a few millimetres with two scalpels equipped with new blades (or fine scissors).

(2) It is then washed three times with Dulbecco PBS solution (with calcium).

(3) The fragments are placed in an Erlenmeyer flask on a magnetic shaker. Trypsin is then added as a solution of 25 mg/l. in PBS buffer and the preparation is agitated in the incubator at 37° after stoppering the Erlenmeyer flask. This pretrypsinization stage lasts 10–20 min according to the consistency of the fragments.

(4) The trypsin solution is then replaced by fresh solution. Agitation is continued and the trypsin replaced every 10–15 min. The solution that has already been used is transferred at each change to a sterile centrifuge tube placed in an ice bath.

(5) Trypsinization usually lasts from 1 to 4 hr according to the tumour studied.

(6) The solutions kept in an ice bath are centrifuged at the end of trypsinization for 5 min at 800 r.p.m.

(7) Wash 3 times in a solution of caseine hydrolysate of the Pasteur Institute or in another culture medium (199, for example).

(8) The sediment is then suspended in 1 or 2 ml casein hydrolysate or medium 199 depending on the volume of this sediment.

(9) Place the culture in test tubes with coverslips containing the following medium: 2 parts caseine hydrolysate or medium 199 and 1 part human AB serum so as to give a concentration of 5×10^5 cells per ml.

(10) The tubes are then placed in the incubator at 37° after adjusting the pH by means of a mixture of 5% CO_2 in air until a yellow–orange colour is obtained.

(11) Cell multiplication occurs after periods greatly varying from one tumour to the other. Renew the medium on average every 3 days.

(12) Prepare the coverslips according to the standard technique: allow 25 min for swelling of the cells. The supernatant is recovered separately and treated as a blood culture (cf. § 4).

B. AUTORADIOGRAPHIC TECHNIQUE

Tritium-labelled thymidine permits the study of DNA synthesis in living cells. Since this substance is exclusively incorporated into deoxyribonucleic acid, the chromosomes synthesized while the medium contains tritiated thymidine will become radioactive. This feature is detected by the technique, now routine, of autoradiography; a sensitive plate placed in direct contact with the preparation is exposed to the β-ray emissions of tritium and grains of silver form which overlie the areas containing tritiated thymidine. This technique enabled Taylor *et al.* (1957) to study the duplication of chromosomes in living cells.

It is easy to see that if tritiated thymidine is present in the medium for a fairly short time it will be possible to study the rate of synthesis of DNA in each chromosome. If a chromosome has entirely completed its synthesis at the moment when tritiated thymidine is added to the medium it will not incorporate thymidine into its DNA (as this is already synthesized) and will thus not be labelled. On the other hand, if synthesis is still in progress tritiated thymidine will be incorporated into DNA and the chromosome will be "labelled".

By thus varying the exposure times it becomes possible to demonstrate chromosomes which label early and those which label late. The X chromosome would appear to belong to the latter group or, to be more exact, one of the two X chromosomes of the female (German III, 1962; Gilbert *et al.*, 1962; Moorhead and Deffendi, 1963). Moreover, this tendency towards late labelling of the X chromosome is thought to correspond to identical labelling of the Barr body (Atkins *et al.*, 1963a). This autoradiographic technique is now in common use and has been employed by various workers as a means of identifying certain pairs (Schmid, 1963) although according to Lima de Faria *et al.* (1962) autosomal pairs are not synchronous.

To be more precise, the special features of the late-labelled X chromosome have been widely studied both in cases of aneuploidy (Rowley *et al.*, 1963; Mukherjee, 1963a; Grumbach *et al.*, 1963) and in the case of structural rearrangements of the X chromosome. Since the long arm of this chromosome is labelled particularly late, this technique may be employed to identify iso-X chromosomes (Muldal *et al.*, 1963; Taft and Brooks, 1963; Giannelli, 1963; Lindsten, 1963a).

The body of evidence gathered with this technique will be presented in the chapters on sex aberrations.

C. TECHNIQUES OF OBSERVATION

Obtaining well-dispersed metaphases by careful application of the techniques described is only the prelude to analysis proper. Because of the paramount importance of microscopic observations and the time which must be spent on these investigations, it is absolutely essential to have a binocular microscope with objectives $\times 8$, $\times 16$, $\times 40$ and $\times 100$ (apochromatic with immersion). Moreover, photographic analysis, which is absolutely essential, requires standard equipment preferably 24×36 film. The use of photographic plates is practically excluded because of the number of negatives necessary.

Attempts at electron microscopic study of human chromosomes run into great difficulties. The first results obtained by Barnicot and Huxley (1961) barely improved the resolving power as compared with the ordinary microscope. However, with the development of new techniques, it may in future be possible to use the electron microscope. Figure 2.2 taken by Dr. Jean de Grouchy shows that the detection of the fine structure of the chromatids is, for the time being, at the limits of possibility.

If one is equipping a laboratory from scratch one should select a microscope

with incorporated photography, preferably with a system of automatic time exposure selection. Such a device gives negatives of constant quality and makes photomicrography a routine matter.

Although study of human chromosomes scarcely differs from other microscopic techniques, it seems useful to specify the following details.

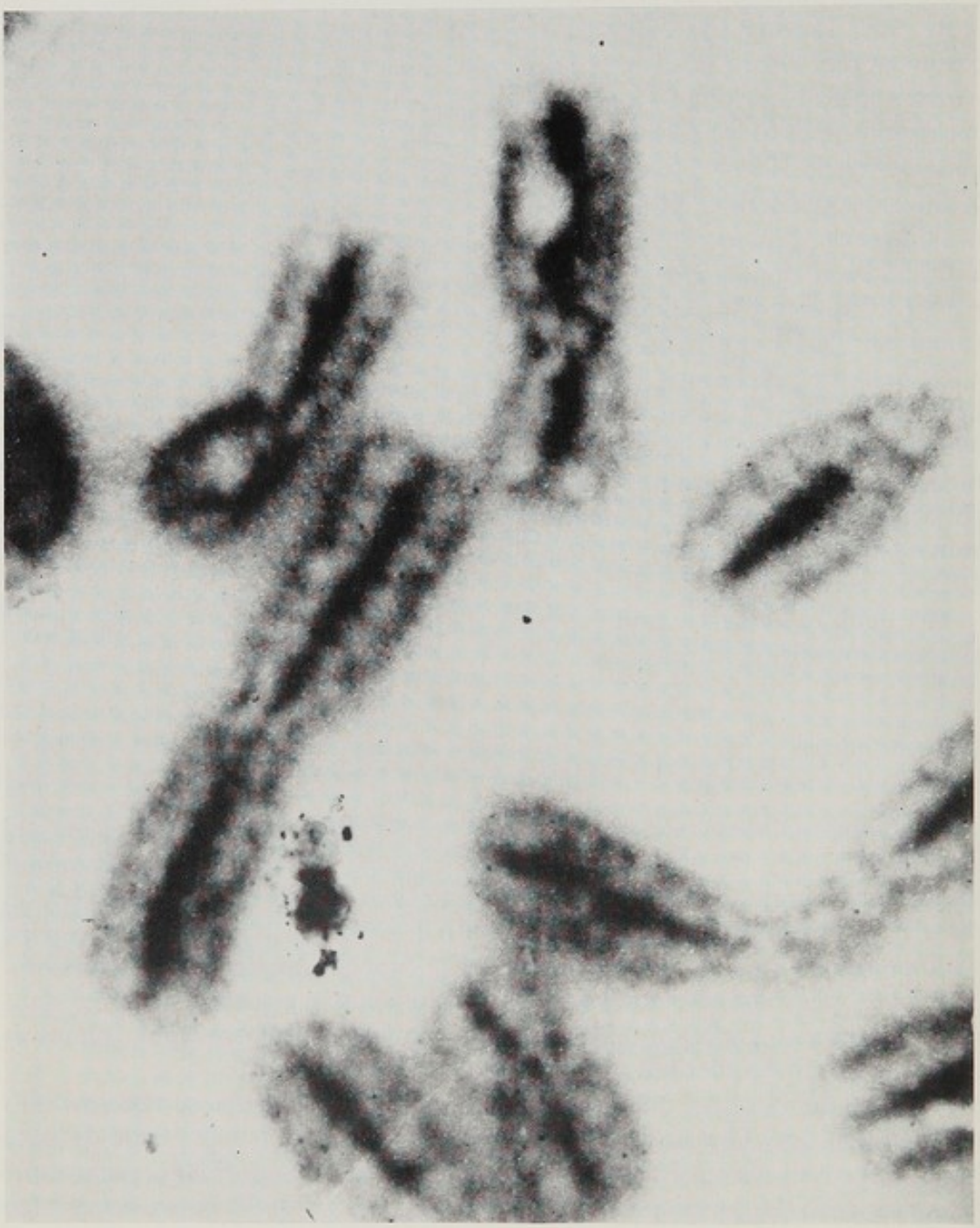


FIG. 2.2. Human chromosomes examined in the electron microscope. Mag. = 8000 × (approx.). Electromicrogram obtained by J. de Grouchy and K.H. Hollmann and F. Haguenau and W. Hellmann (Collège de France, Laboratory of Experimental Medicine).

(a) Detection of favourable mitoses

This detection is made with a dry objective ×8 in order to run quite rapidly through the preparations while permitting a first diagnosis of the "quality" of the mitoses.

With magnification ×16 or ×40 the really favourable cells are selected and then examined with oil immersion.

For any cell examined it is necessary to count the number of chromosomes and to make certain of the integrity of the karyotype.

(b) Counting technique

If the observer has had a great deal of experience counting on sight may be a real aid but it is subject to errors which are difficult to detect. A simple method consists in observing the cell through a reticulum such as that of photographic oculars. One thus arbitrarily delimits "fields" in which it suffices to count the chromosomes. For greater certainty it is possible to use a small counter of a type employed for blood counts which tells us the total number only after the end of the examination. Practice shows that it is preferable to count the chromosomes in small groups of three in order to detect more easily those which have already been counted.

As against its rapidity this technique of counting by sight has serious defects: no control is possible unless a new count is made and there is no documentation. We prefer the drawing technique.

(c) Drawing

The classical free-hand drawing obviously still can be used but for greater precision a camera lucida may be very helpful.

However, we prefer a personal technique which requires the use of a projection lens (Zeiss type, for example) or a standard frosted glass. A sheet of cellophane† is fixed by four pieces of adhesive tape to the frosted surface. Observation of the chromosomes through the cellophane is not impeded and it becomes possible to trace each of them by simply using an ordinary black-ink fountain pen or a hard pencil.

The possibility of turning over the cellophane once the drawing is completed eliminates the difficulty of image reversal produced by certain types of projections.

The advantages of drawing onto cellophane are threefold:

(1) it permits certain and rapid counting and by visual control allows precise identification of chromosomes easy to identify (21, 22, Y, 13–15, etc.) and if one is dealing with a structural anomaly the abnormal chromosome may be directly emphasized on the drawing;

(2) the drawing in itself constitutes a document which may be kept and its informative value is almost identical with that of a photograph; and

(3) if care is taken to plot the Cartesian coordinates of the cell drawn and the number of the slide observed, it becomes possible to check previous work very

† The sheets of cellophane sold as jam jar covers are perfectly suitable for this purpose.

rapidly. When the cell is again projected on the focusing screen the cellophane is replaced in such a way that the drawing coincides with the projection. It is then possible to spot at once any error and verification in this way is extremely rapid.

The time spent on these drawings is a matter of a few minutes per cell and hence appreciably longer than for counting by eye but we consider that its advantages make it preferable. Any cell "counted" in our laboratory is, in fact, drawn or photographed.

(d) Photography

In addition to direct analysis by eye or drawing, it is essential to obtain excellent photomicrographs. As stated above, the use of a microscope with an automatically controlled time exposure enormously facilitates the work of the researcher. However, the point must be made that any device may be used which focusses correctly but at the cost of some uncertainty of the exposure times.

(e) Choice of cells

Since the photographs are destined for karyotyping after each of the chromosomes has been individually cut out, it is legitimate to impose a severe selection on their quality. Apart from the particular cases where aberrations are the subject of the study, one should systematically not select cells with defective morphology: broken chromosomes, indistinct or jagged chromosome contours and obvious aneuploidy expressed in loss of whole packets of chromosomes.

(f) Filming

The quality of the staining is a primary condition for the quality of the negatives and that is why we prefer staining with Unna's blue which gives excellent contrast. When the contrast is not distinct enough phase contrast may be useful. However, a certain loss in definition of the image during phase contrast and the insufficient planeity of modern optics limits its use when the preparations are properly stained. Its only merit is that it enables one to salvage imperfect material.

However, it should be pointed out, by way of curiosity, that it is perfectly possible to photograph unstained preparations with phase contrast. Unfortunately, this technique which would have the theoretical advantage of eliminating a number of possible artefacts is far from supplying documents as satisfactory as those obtained with staining.

The quality of the emulsion used is highly important. As the image received is essentially composed of purple rods against a bright background a high gamma film is indispensable. A film with a very fine grain and hence, very slow, should be chosen. Kodak panchromatic Microfile film meets these requirements. It is necessary to "expose" for quite a long time even if one has a powerful light source. At the magnifications used, the mere opening of the shutter causes a vibration such that the image of an exposure at 1/50th sec, for example, has every chance of being blurred by superpositioning of two images. An exposure of the order of 1–2 sec practically eliminates these vibrations which only lasting 1/100th of the exposure time do not have time to mar the final image.

(g) Enlarging

The photograph should be taken preferably on 24 × 36 mm film and the optimum enlargement on the negative is of the order of ×450–500. Secondary enlargement is necessary and we consider that a final magnification of ×3500 to 4000 on the developed photograph represents an acceptable compromise between the material difficulties and the need for effective definition and precise comparison of the different elements of the chromosomal set.

Since photography is an integral part of chromosome identification, it is necessary to do the photography in the laboratory itself.

(h) Number of cells examined

We now have to estimate the number of cells which must be examined before we can reach a conclusion on the karyotype of an individual.

In the absence of mosaics it seems legitimate to consider that a few "perfect" cells the karyotype of which has been established without ambiguity warrant a firm conclusion. The problem thus boils down to the probability of the detection of a mosaic.

Detection of cell variants

To tackle this problem we may at first use Table 2.1 which indicates the probability of detection of at least one exceptional cell as a function of their frequency, on the one hand, and the number of cells examined, on the other.

TABLE 2.1

Theoretical probability of detection of at least one "aberrant" cell as a function of the number of cells inspected and the frequency of exceptional cells in the sample studied

Frequency of exceptional cells	Number of karyotypes									
	2	4	8	16	32	64	128	256	512	1024
0·5	0·75	0·94	0·996	0·999	0·999	1	1	1	1	1
0·4	0·64	0·87	0·98	0·999	0·999	1	1	1	1	1
0·3	0·51	0·76	0·94	0·997	0·999	1	1	1	1	1
0·2	0·36	0·59	0·83	0·97	0·999	1	1	1	1	1
0·1	0·19	0·35	0·57	0·81	0·97	0·998	0 999	1	1	1
0·01	0·01	0·04	0·08	0·15	0·28	0·48	0·72	0·92	0·994	0·999
0·001	0·002	0·004	0·008	0·02	0·04	0·06	0·12	0·23	0·64	0·87

For 0·001 the limit 0·01 is virtually reached with 4000 cells (0·985) and 0·9998 with 8192 cells.

Such a calculation implies that the experimental variance, that is, production of abnormal karyotypes by artefact, is almost nil. Such a state of affairs is virtually realized in fibroblast culture on a coverslip since for cells never having been manipulated in the open the risks of loss or gain of chromosomes are reduced to a minimum.

Even under these optimal conditions, far from being achieved in blood cultures in liquid medium, Table 2.1 shows that a mosaic making up less than 10 per cent is very difficult to detect.

Diagnosis of the mosaic proper

The preceding stage enables us to select only cultures in which one cell in 16 (at least) is exceptional. The statement that a mosaic exists is based on the following observation: all the variants are identical amongst themselves, that is, they all show the same karyotype.

If we then take as the criterion of detection observation of at least three cell variants identical amongst themselves, we arrive at Table 2.2.

TABLE 2.2

Theoretical probability of detection of at least three "aberrant" cells as a function of the number of cells inspected and the frequency of exceptional cells in the sample studied

Frequency of exceptional cells	Number of karyotypes									
	4	8	16	32	64	128	256	512	1024	2048
0·5	0·313	0·863	0·997	0·999						
0·4	0·189	0·684	0·983	0·999		Region of certain detection				
0·3	0·083	0·449	0·905	0·999						
0·2	0·048	0·217	0·620	0·934	0·999					
0·1	0·008	0·047	0·217	0·620	0·954	0·999				
0·01	0·000	0·000	0·000	0·004	0·027	0·138	0·471	0·885	0·998	

It will be seen that a sample of 16 cells permits excellent detection of even small mosaics (0·1) and that 64 cells enable us to "confirm" these mosaics. In mosaics of the order of 0·01, detection is possible but confirmation is practically beyond the material resources of a laboratory.

This purely theoretical presentation of the problem of the size of sampling raised by the existence of mosaics seems to us essential since this is one of the most intricate problems of practical cytogenetics.

In conclusion, it may be considered that 16 cells represent a "reasonable" sample for a normal subject. In consequence, we systematically adopt the following course: for each individual six cells are photographed and analysed, two among them being cut up for inspection of the karyotype. Ten other cells are drawn on cellophane according to the protocol described or are photographed and then analysed.

If the 16 cells thus checked all show a normal set the individual is considered normal, not mosaic. This latter statement is limited by three factors:

(1) homogeneity only applies to the fraction of tissue actually examined and extrapolation to the whole individual is only tentative;

(2) absence of a mosaic is only probable for a level of heterogeneity higher than a certain value (here 0·1);

(3) the possibility of differential survival *in vitro* cannot be ruled out (cf. Chapter 8).

CHAPTER 3

Normal Human Karyotype

THE studies published to date (cf. Chapter 1) permit us to conclude that the number and morphology of the various elements of the karyotype of normal individuals are constant. Therefore it is essential to use an international classification to permit exchange of information.

A group of researchers meeting in Denver in the spring of 1960 worked out a numerical classification exclusively used at the present time and representing a reference code.

This convention is based on two principles:

(1) The chromosomes are classed in order of diminishing size, the largest one bearing the number 1 and the smallest the number 22; recognition of each of them is based on the relative length of the element, on the one hand, and the position of its centromere on the other.

(2) Within each group the chromosomes are arranged in descending order of size.

The two sex chromosomes X and Y keep their classical lettering to avoid needless confusion.

The Denver document defines seven groups which we shall briefly recall. The interest of the groups, numbered from the first and the last number of the sequence (for example, group (1–3) for large chromosomes) lies in the fact that any normal chromosome may be formally assigned to a given group. On the other hand, even within a given group, difficulties of identification may arise which are made even worse if the quality of the preparation studied is not optimum.

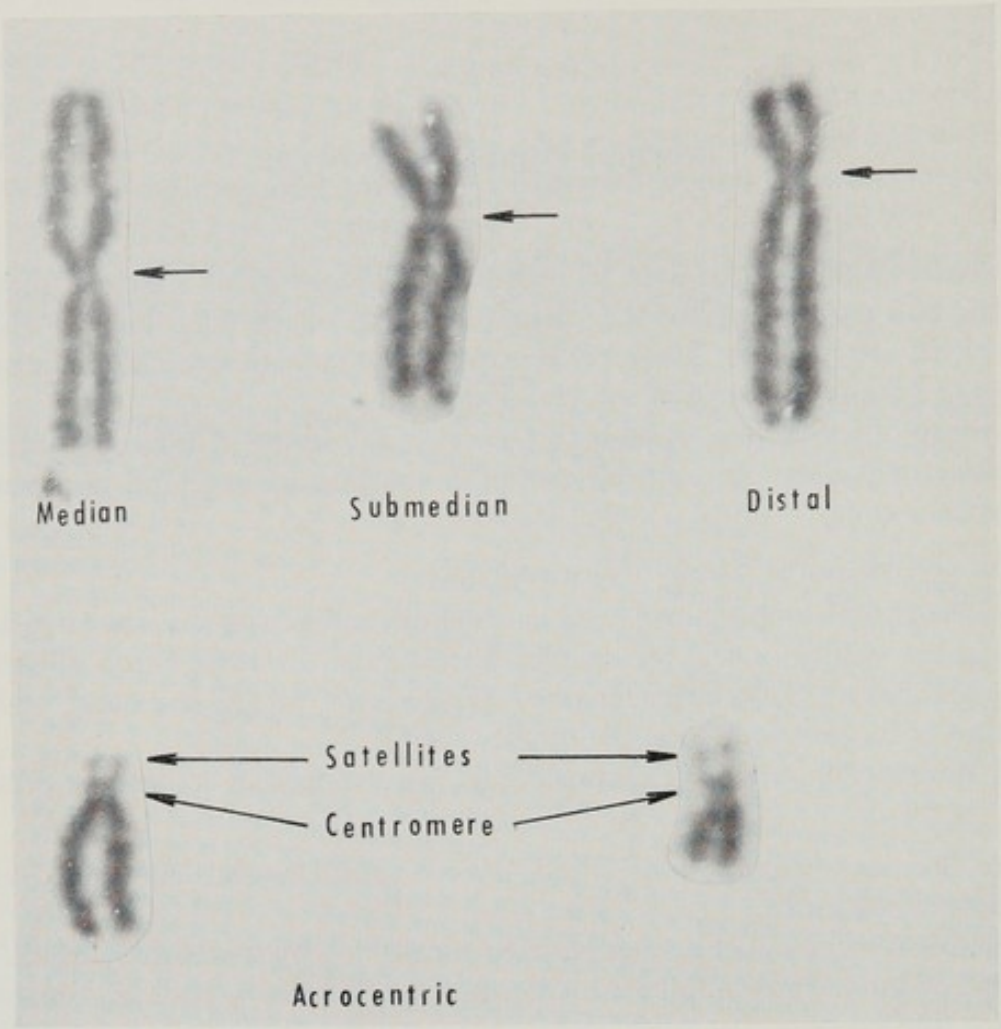
Conspectus of human, mitotic chromosomes

(A) Group (1–3)—large chromosomes with approximately median centromeres. The three chromosomes are readily distinguished from each other by their size and centromere position.

(B) Group (4–5)—large chromosomes with distal centromeres. These two chromosomes are difficult to distinguish, but chromosome 4 is slightly longer.

(C) Group (6–12)—medium-sized chromosomes with submedian centromeres. The X chromosome resembles the longer chromosomes in the group, especially chromosome 6, from which it is difficult to distinguish it. This important group is the one which presents major difficulty in the identification of individual chromosomes.

TABLE 3.1
Position of the centromere



(D) Group (13–15)—medium-sized chromosomes with nearly terminal centromeres ("acrocentric" chromosomes). Chromosome 13 has prominent satellites on the short arms. Chromosome 14 has small satellites on the short arms. No satellites have been detected on chromosome 15 (or almost none).

(E) Group (16–18)—rather short chromosomes with approximately median centromeres (in chromosome 16) or submedian ones.

(F) Group (19–20)—short chromosomes with approximately median centromeres.

(G) Group (21–22)—very short acrocentric chromosomes. Chromosome 21 has satellites on its short arms. The Y chromosome is similar to these chromosomes.

According to Patau (1960), it is possible to use the lettering A, B, etc., corresponding to each group to simplify writing (see Chapter 7).

Analysis of the human karyotype

This analysis of the human karyotype is essentially based on the fact that one may define a chromosome by two parameters, its length and the position of its centromere. These parameters related to the physical structure of the chromosome are theoretically fixed but show an important variance arising from two main causes: the technique of preparation and the physiological state of the cells.

Without considering these variations for the moment, we shall look at the two techniques of analysis immediately applicable, the method of pairs and the topographic method.

Method of pairs

Since each chromosome pair consists of a chromosome coming from the father and a chromosome coming from the mother a simple postulate, amply confirmed by experience, is that the two homologous chromosomes should show a greater resemblance between themselves than two non-homologous chromosomes.

It is thus possible to assemble two by two the 46 elements of the karyotype to form 23 pairs (in the female) each of which will be composed of two elements the length and position of the centromere of which will be as similar as possible.

These pairs will then be arranged in groups and distributed according to their length within the groups.

This simple method gives very faithful results and it is quite remarkable to note the highly concordant results in the hands of very different workers using different techniques on tissues of the most diverse origin.

The second stage is to describe by one number the length of each chromosome and by a second, the position of its centromere. The accumulation of many karyotypes can then be used to calculate a mean value for these two parameters and to say, for example, chromosome 1 has such a length and its centromere is found in such a position.

These parameters are evaluated as follows:

(a) Length of a chromosome

The precise measurement of the actual length of a chromosome is relatively easy either by observation with a micrometric ocular or by measuring a photographic image, whose enlargement is known precisely. However, the length of a chromosome varies within very wide limits as a function of the stage of mitosis (from 20 μ to 8 μ for example, for chromosome 1 between the prophase and the metaphase). It also varies with the degree of uncoiling of the secondary spiral due to the action of the hypotonic shock.

It is therefore desirable to choose a parameter relatively independent of these two variables, the relative length, that is, the actual length of a pair related to the total length of all the other pairs.

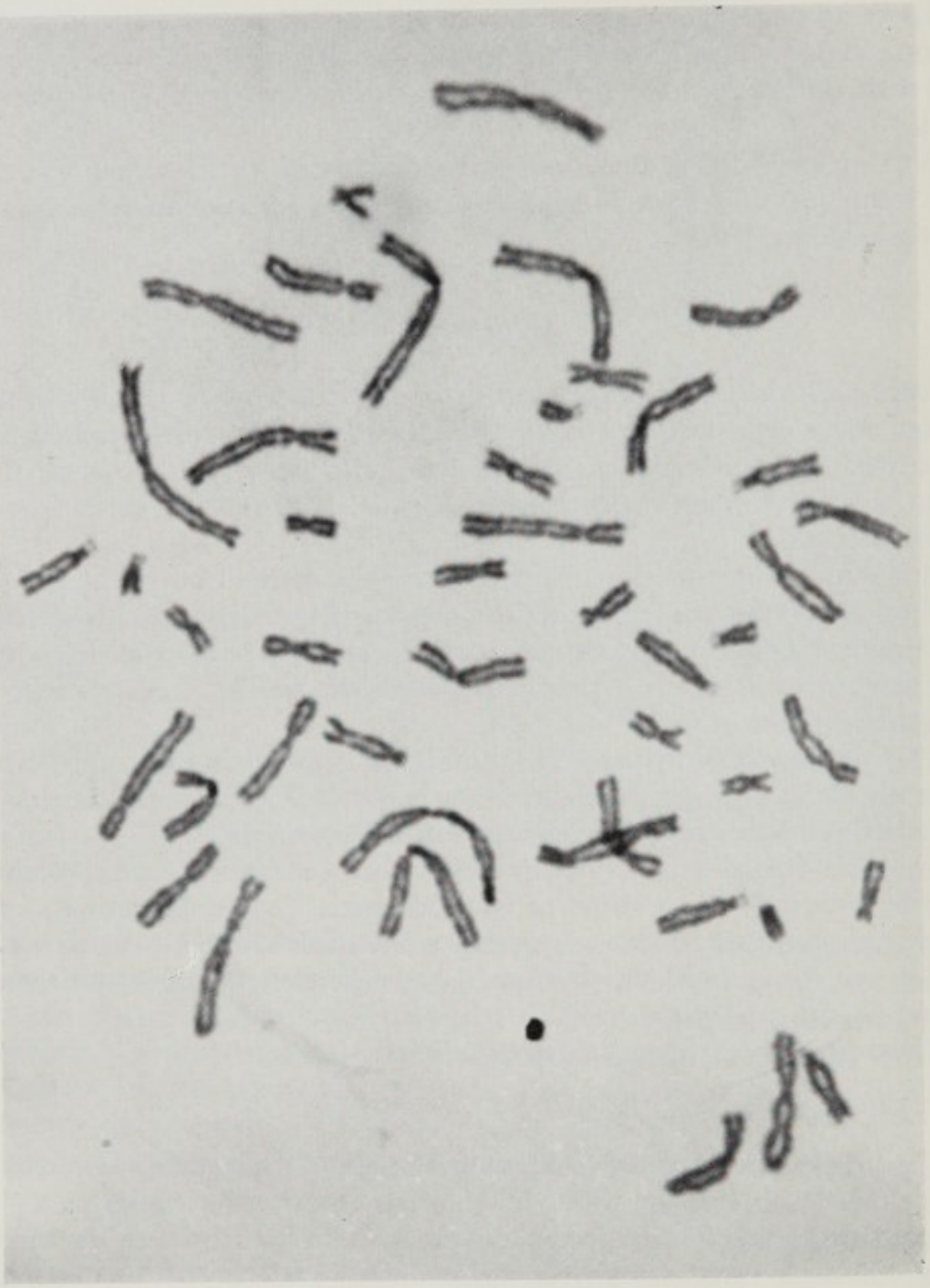


FIG. 3.1. Normal male. Cell with 46 chromosomes.

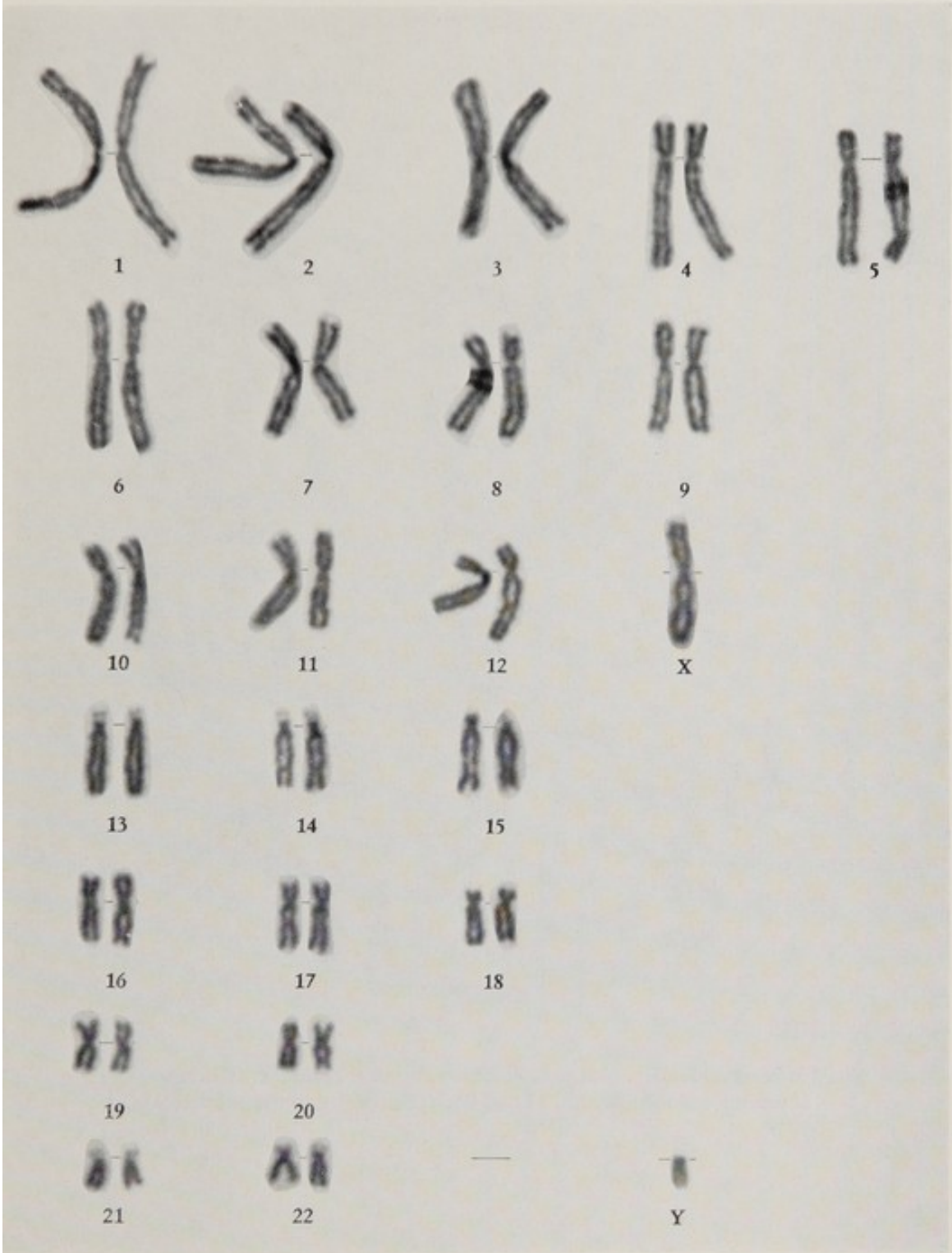


FIG. 3.2. Normal male. Karyotype 44A + XY.

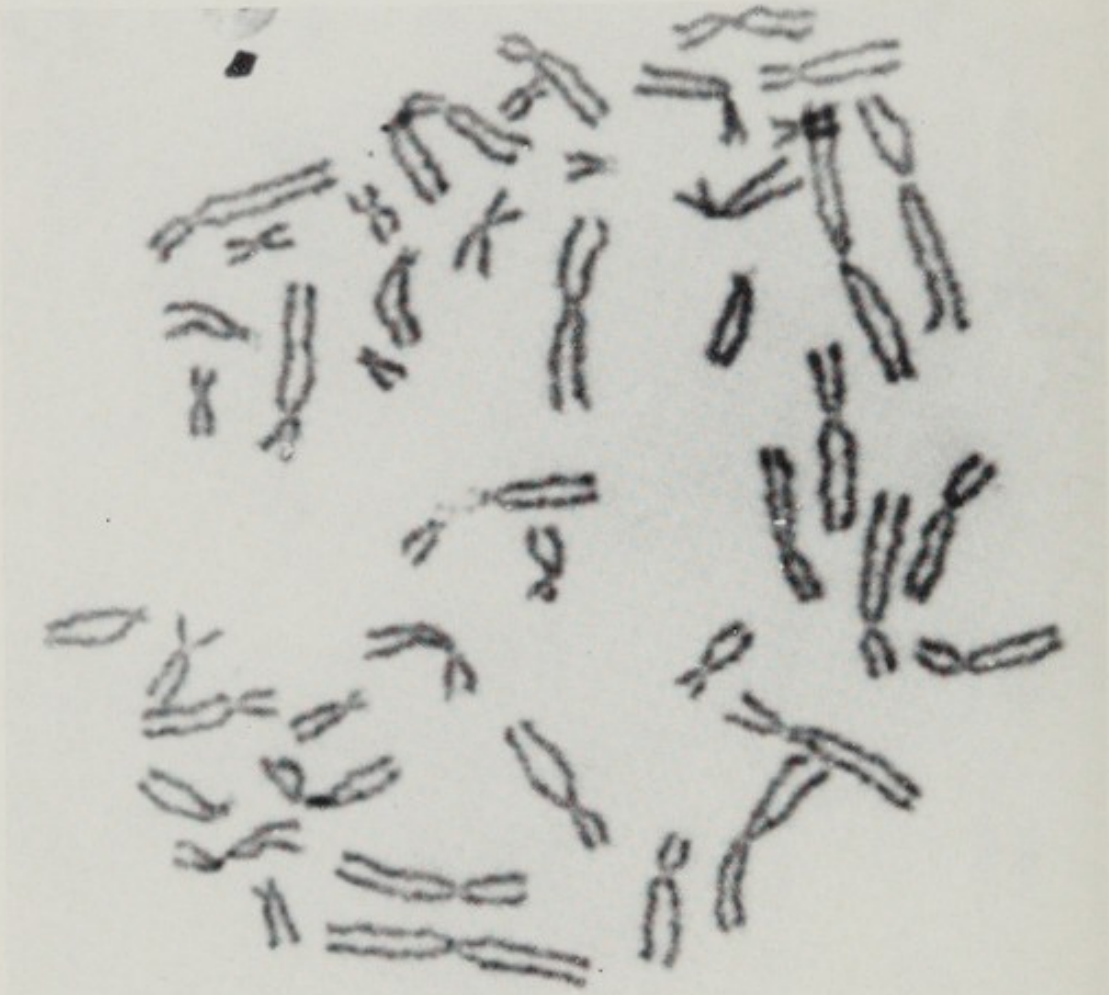


FIG. 3.3. Normal female. Cell with 46 chromosomes. Note the association of two acrocentric chromosomes, 13 and 21, at the centre of the preparation.

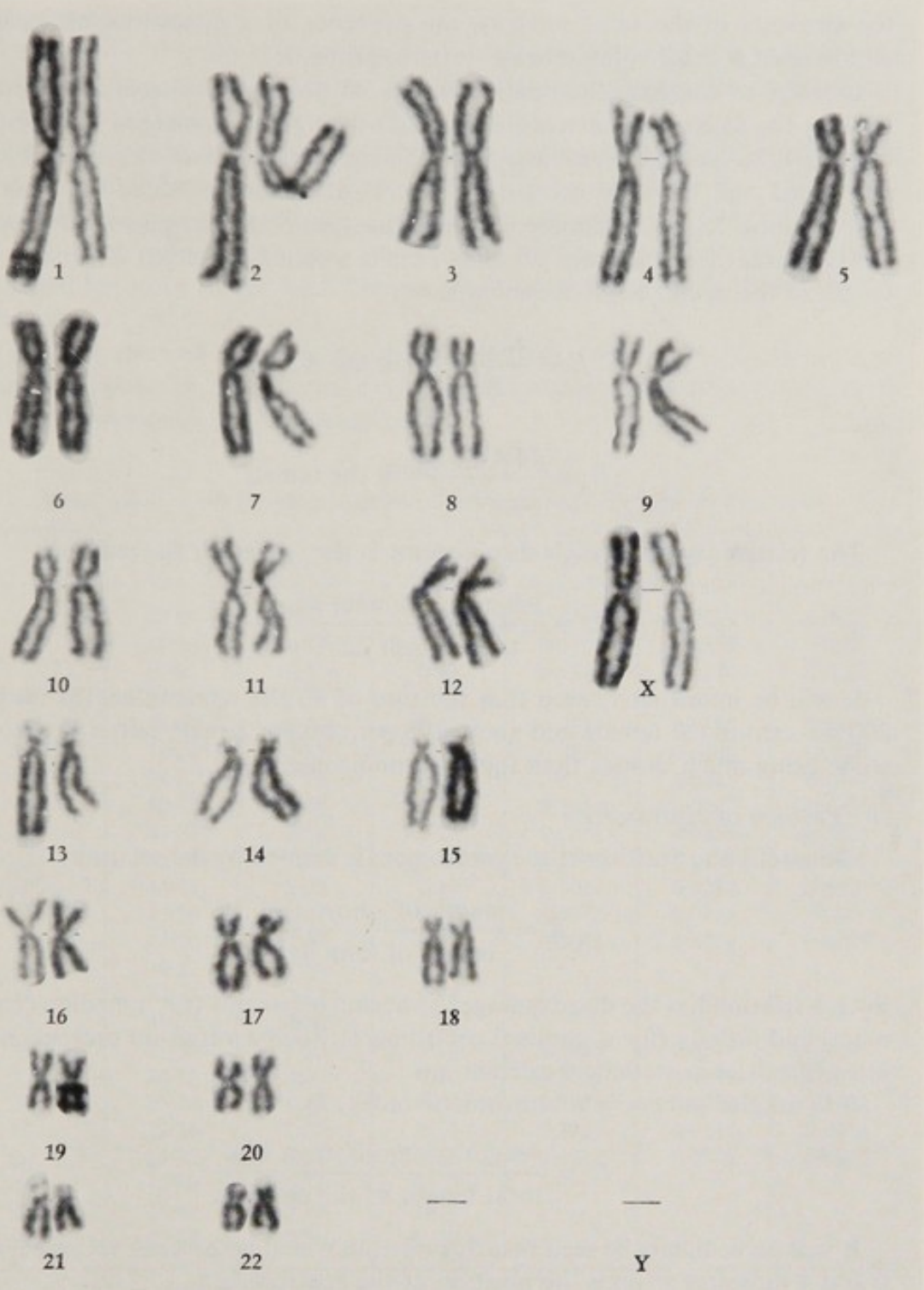


FIG. 3.4. Normal female. Karyotype 44 A + XX.

The synchronous evolution of all the chromosomes of the same nucleus suggests that they all contract at the same time and in the same proportions. Likewise, the effect of hypotonic shock must be more or less the same for all the elements of the same nucleus, the existence of a concentration gradient within such a small volume being quite improbable.

In order to compare the relative lengths of the chromosomes in males and females the following convention is chosen: on greatly enlarged photographs ($\times$ 4000) the lengths in millimetres of all the autosomes of the same nucleus are added and the total divided by two. To this value is added the length of chromosome X if it is a male and half the sum of the lengths of the two X chromosomes for a female. We thus obtain a standard length E equal to the length of the neuter haploid genotype or:

$$E = \frac{44\,\mathrm{A}}{2} + \mathrm{X} \text{ in the male}$$

and

$$E = \frac{44\,\mathrm{A} + 2\mathrm{X}}{2} \text{ in the female}$$

The relative length of each chromosome is then given by the relation:

$$L = \frac{\text{length of element studied}}{\text{total length } 22\,\mathrm{A} + \mathrm{X}}$$

It will be immediately seen that the sum of all the relative lengths must be 200 per cent in the female and about 196 per cent for a male cell, a Y chromosome being much shorter than the X chromosome.

(b) Position of centromere

Classically the position of the centromere is defined by the relation:

$$R = \frac{\text{length of short arm}}{\text{length of long arm}}$$

Such a relation has the disadvantage of varying between 1 (for a median centromere) and infinity (for a terminal centromere); such a variation presents needless difficulties in statistical calculations.

It is simpler to choose a centromeric index, Ic:

$$Ic = \frac{\text{length of the shortest arm}}{\text{total length of the element}}$$

It will immediately be seen that this relation which necessarily varies between 0 and $\frac{1}{2}$ indicates at once the position of the centromere.

An Ic of 0·5 corresponds to a median centromere (situated at half the length of the chromosome), an index of 0·25 to a distal centromere (situated at a quarter of the length) and a centromere of index 0·1 to a nearly terminal centromere (acrocentric chromosome).

In all that follows it is assumed that the satellites, if they exist, are not taken into consideration in the measurement of the chromosomal length because of their extreme variability. Thus, the length is only measured on the chromosome arms proper.

The analysis of many cells then permits a calculation of a mean value for the length of a given chromosome and also for the centromeric index. A given pair may be thus defined by these two mean values.

The results of measurements made at the Institut de Progénèse on 100 cells (50 male and 50 female) are given in Tables 3.2, 3.3 and 3.4.

It should be noted that these values refer to cells not treated with colchicine and the slight differences which may be seen between these figures and those published by other workers (cf. Denver classification) are probably due to this difference.

This first method of analysing the human karyotype, however satisfactory it may be in practice, does not entirely solve the problem of precise identification of the chromosomes for two reasons.

TABLE 3.2

Mean values of the length (L) and the centromeric index (Ic) for 50 male cells

	$\bar{L}$	σL	$\sigma\bar{L}$	$\bar{I}c$	σIc	$\sigma\bar{I}c$
1	88·68	5·38	0·54	0·4767	0·0216	0·0022
2	82·36	4·41	0·44	0·3881	0·0258	0·0026
3	67·91	4·47	0·45	0·4512	0·0244	0·0025
4	62·59	3·27	0·33	0·2715	0·0151	0·0015
5	58·60	2·96	0·30	0·2530	0·0185	0·0019
X (50)	58·04	2·57	0·26	0·3724	0·0200	0·0020
6	54·52	2·73	0·27	0·3592	0·0215	0·0022
7	50·18	2·88	0·29	0·3655	0·0248	0·0025
8	47·04	2·58	0·26	0·3045	0·0289	0·0029
9	45·77	2·21	0·22	0·3696	0·0331	0·0033
10	44·71	2·00	0·20	0·3020	0·0284	0·0029
11	43·19	1·97	0·20	0·3742	0·0311	0·0031
12	43·20	2·07	0·21	0·3081	0·0250	0·0025
13	32·52	2·59	0·26			
14	31·93	2·82	0·28			
15	31·23	2·40	0·24			
16	29·88	1·90	0·19	0·3983	0·0347	0·0035
17	28·68	2·02	0·20	0·3054	0·0391	0·0039
18	25·40	1·92	0·19	0·2821	0·0322	0·0032
19	22·34	1·58	0·16	0·4321	0·0334	0·0034
20	21·32	1·51	0·15	0·4332	0·0370	0·0037
21	15·40	1·73	0·17			
22	15·67	2·30	0·23			
Y (41)	15·70	2·28	0·34			

N.B. The figure 41 for Y takes into account the presence of nine haplo X cells in our sample.

Lettering: σL = standard deviation of the distribution of L

$\sigma\bar{L}$ = standard error of the mean $\bar{L}$

σIc = standard deviation of the distribution of Ic

σIc = standard error of the mean $\bar{I}c$

TABLE 3.3

Mean value of the length (L) and centromeric index (Ic) for 50 female cells

	$\overline{L}$	σL	$\sigma\overline{L}$	$\overline{Ic}$	σIc	$\sigma\overline{Ic}$
1	87·60	3·00	0·30	0·4767	0·0209	0·0021
2	82·54	5·24	0·53	0·3879	0·0242	0·0024
3	67·32	3·78	0·38	0·4501	0·0234	0·0023
4	62·44	3·33	0·34	0·2728	0·0187	0·0019
5	59·20	4·26	0·43	0·2727	0·0209	0·0021
X (100)	56·60	3·78	0·38	0·3726	0·0246	0·0025
6	52·80	3·34	0·34	0·3621	0·0256	0·0026
7	49·02	2·58	0·26	0·3688	0·0246	0·0025
8	46·86	2·44	0·25	0·3062	0·0268	0·0027
9	45·90	2·04	0·21	0·3682	0·0306	0·0031
10	45·03	1·81	0·18	0·2965	0·0250	0·0025
11	43·69	2·14	0·22	0·3776	0·0273	0·0027
12	43·25	1·81	0·18	0·2994	0·0223	0·0022
13	32·69	2·25	0·23			
14	32·18	2·48	0·25			
15	32·30	2·51	0·25			
16	30·03	2·09	0·21	0·4105	0·0419	0·0042
17	29·14	2·32	0·23	0·3178	0·0432	0·0044
18	25·53	1·81	0·18	0·2888	0·0357	0·0036
19	23·09	1·64	0·16	0·4401	0·0322	0·0032
20	21·65	1·58	0·16	0·4339	0·0289	0·0029
21	15·86	1·76	0·18			
22	15·66	2·28	0·23			

Lettering: σL = standard deviation of the distribution of L
$\sigma\overline{L}$ = standard error of the mean $\overline{L}$
σIc = standard deviation of the distribution of Ic
$\sigma\overline{Ic}$ = standard error of the mean $\overline{Ic}$

(1) Intrinsic variance due to pairing

While there is no ambiguity for chromosomes 1, 2 and 3, for example, this is certainly not true for group 4 and 5.

We see, in effect, that chromosome 4 ($L = 62{\cdot}5$) is considerably longer than 5 ($L = 58{\cdot}9$) at least, according to Table 3.1. We know that in fact these two chromosomes are very difficult to distinguish from each other and it must be feared that the pairing off method exaggerates the difference (if it exists). In fact, the classification is made by size and chromosome 4 must be longer than 5 by definition; or to be exact, the longest of the two is called No. 4 and the shortest, No. 5.

Let us suppose for a moment, as was also pointed out by Patau (1960), that the four elements of group 4–5 are in fact, homologues, that is, our species is tetraploid for this type of chromosome. Since we pair two by two the chromosomes which most closely resemble each other and since we assign the longest pair the number 4 and the shortest the number 5, any fortuitous extra length (accidental stretching during the drying of the preparation) will be exclusively

TABLE 3.4

Mean value of the length (L) and centromeric index (Ic) for 100 cells (50 male and 50 female)

	$\bar{L}$	σL	$\sigma\bar{L}$	$\bar{I}c$	σIc	$\sigma\bar{I}c$
1	88·14	4·35	0·30	0·4764	0·0214	0·0015
2	82·45	4·84	0·34	0·3880	0·0250	0·0017
3	67·71	4·13	0·30	0·4506	0·0238	0·0016
4	62·51	3·30	0·23	0·2721	0·0170	0·0012
5	58·90	3·59	0·39	0·2728	0·0197	0·0014
X (150)	57·08	3·61	0·26	0·3726	0·0258	0·0018
6	53·69	3·04	0·21	0·3606	0·0236	0·0016
7	49·60	2·73	0·19	0·3671	0·0246	0·0017
8	46·95	2·51	0·18	0·3053	0·0279	0·0019
9	45·83	2·14	0·15	0·3689	0·0319	0·0022
10	44·87	1·92	0·13	0·2992	0·0268	0·0019
11	43·44	2·04	0·14	0·3759	0·0294	0·0021
12	42·72	1·94	0·13	0·3037	0·0238	0·0016
13	32·60	2·42	0·17			
14	32·05	2·66	0·18			
15	32·26	2·46	0·17			
16	29·95	2·01	0·14	0·4044	0·0386	0·0027
17	28·91	2·19	0·15	0·3116	0·0412	0·0029
18	25·46	1·87	0·13	0·2854	0·0340	0·0024
19	22·71	1·64	0·11	0·4361	0·0328	0·0023
20	21·48	1·58	0·11	0·4335	0·0333	0·0023
21	15·63	1·76	0·12			
22	15·66	2·28	0·15			
Y (41)	15·70	2·28	0·34			

This table summarizes the mean values of the relative length (expressed in thousandths) and of the centromeric index for all the 100 cells studied.

Lettering: σL = standard deviation of the distribution of L

$\sigma\bar{L}$ = standard error of the mean $\bar{L}$

σIc = standard deviation of the distribution of Ic

$\sigma\bar{I}c$ = standard error of the mean $\bar{I}c$

related to 4 and accumulation of data will only accentuate this bias. In the extreme case, a statistically significant difference between L_4 and L_5 will be demonstrable even if these two pairs are, in fact, strictly homologous. Such reasoning applies *a fortiori* to the difficult group (6–12) plus X and one may wonder if the classification by pairing off may produce "pairs" which are merely the result of accumulated artefacts.

(2) Exact calculation of the relative length

Another defect of the preceding statistics is that the reference value 22A + X can be directly estimated only in the female. In this case, 22A + X is equal to half the length of all the chromosomes.

In the male, the problem is much more complex since before any calculation it is necessary to identify the X and Y chromosomes. In fact, it is necessary to

exclude the chromosome Y from the autosomes and to add the entire length of X to half the total length of the autosomes to obtain the reference length 22A + X.

It will be seen immediately that since the identification of X is very difficult and that of Y sometimes tricky, a systematic error may exist in all the relative lengths in a male cell.

The method of pairs enables us to identify the X chromosome only in the male and not in the female.

All these difficulties may be removed by a statistical procedure which we shall now describe.

Topographic method

This very simple method rests on the following postulate: there is a fixed relation between the relative length of a chromosome and the position of its centromere and this relation is identical for all the chromosomes of the same type (same number) whatever the cell in which they are examined.

This new postulate is basic for the morphological study of chromosomes since it expresses the fact that the chromosome possesses a definite structure identical for all cells and that the centromere occupies a fixed and unchangeable position (rearrangements apart) in this structure.

It is quite clear that the whole theory of cytogenetics would be untenable if such a postulate were manifestly false and it is therefore legitimate to assume that this postulate is a "minimum postulate".

Determination of the "site" of a chromosome

After measuring the relative lengths L and position of the centromere Ic as before (the data used in this chapter were established in 1962 for 100 cells (50 male and 50 female)) each individual chromosome is plotted on a karyogram the abscissa of which represents the relative length and the ordinate the centromeric index.

Each chromosome is then represented by a point on this graph, this point being pierced in practice by a dull black pinhead. Therefore, a single point corresponds to any chromosome measured and the aim of the analysis is to estimate if these "points" tend to group themselves in concentration "sites".

From what we have just said on the random variation of length and centromeric index of the chromosomes, none of these points has absolute value. To be more precise, the coordinates of each of them represent an estimate of the site where the ideal chromosome "ought" to be. It is then tempting to relate each observation to a zone of probability centred on and around the coordinates actually measured.

A photographic representation of this statistical concept may be obtained simply, by an original method which we shall briefly describe.

For greater facility these points have been plotted on different karyograms, one for each group of chromosomes.

Case of group 4–5. The distribution of the points (Fig. 3.5) shows from the

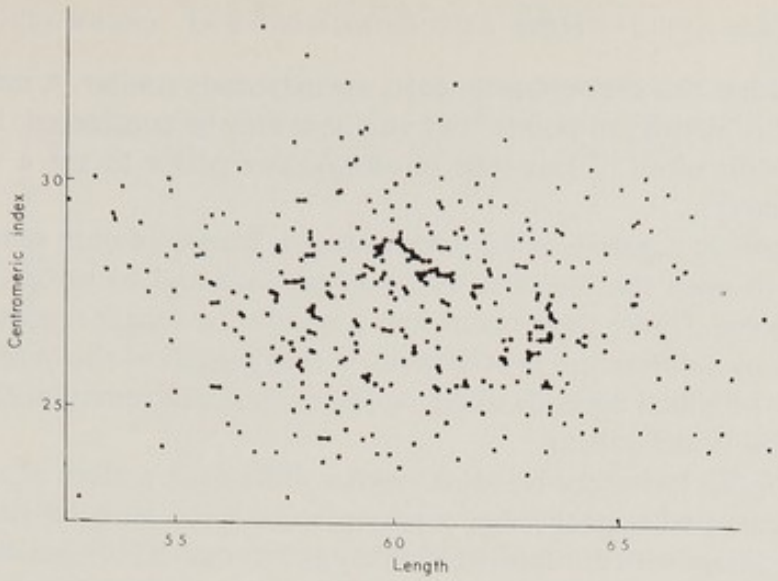


FIG. 3.5

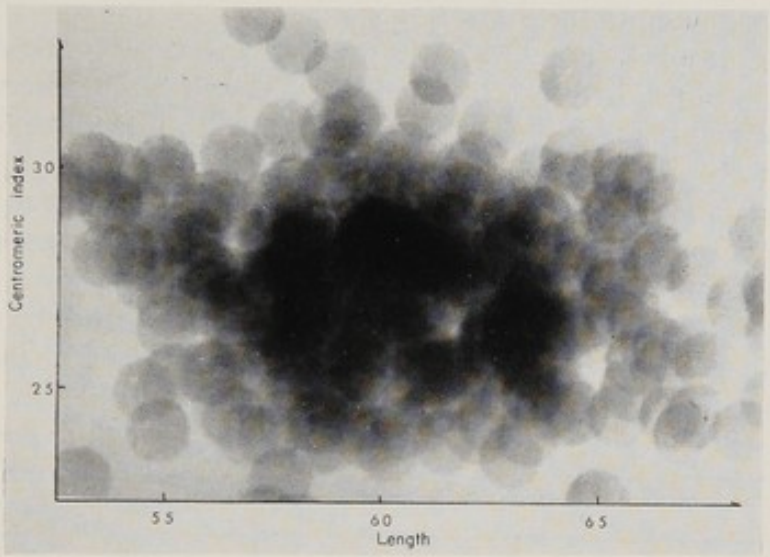


FIG. 3.6

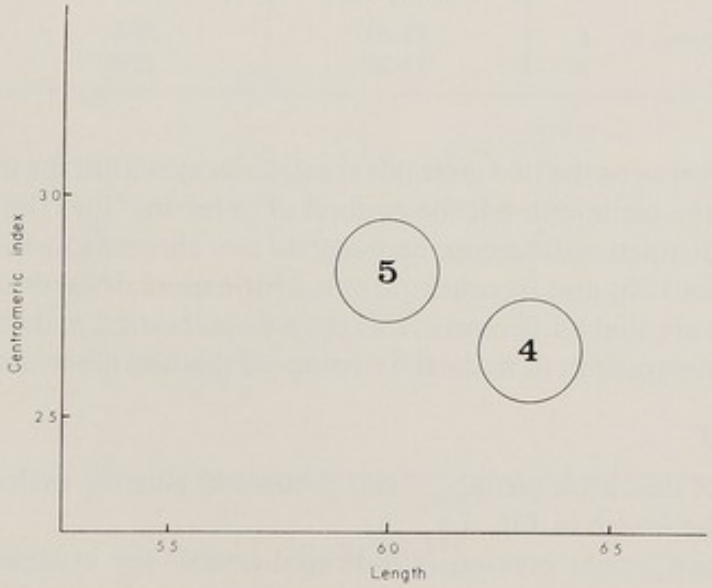


FIG. 3.7

outset that these two chromosome pairs are extremely similar. A mathematical analysis of the "density of points" per unit area may be considered; however, in view of the difficulties of this type of analysis we prefer to use a very simple optical procedure.

A photographic negative of the point scatter is projected onto sensitive paper taking care to open the objective as far as possible and to bring the enlarger well out of focus. Under these conditions a point is no longer represented by its "image" but by a diffraction spot centred on the theoretical site of the point and the diameter of which depends solely on the numerical opening of the objective and the extent of defocusing.

A defect of this technique is that it yields a diffraction pattern of more or less uniform intensity whereas the ideal representation of the probable site of a point should be an image with diminished intensity at the edges (Gaussian distribution). Despite this imperfection the technique of the blurred image has the advantage of giving overlapping images of adjacent points and of summing the quantities of light corresponding to the diffraction spots.

In the case of group 4–5, Fig. 3.6 shows that despite a fairly erratic distribution of the spots, two distinctly marked condensation centres are discernible, one situated at the coordinates $L = 63{\cdot}0$ and $Ic = 26{\cdot}5$, the other at the coordinates $L = 59{\cdot}8$ and $Ic = 28{\cdot}4$.

It is thus reasonable to assume that the first point corresponding to the longest chromosome must be considered as the "site" of chromosome 4, the other point corresponding to the "site" of chromosome 5 (Fig. 3.7).

It is quite difficult to define the statistical significance of this topographical correlation but it is at least interesting to compare these results with the statistical calculation previously made by the pairing-off method.

		Method of pairs	"Blurring" method
Chromosome 4	*L*	62·51	63
	Ic	27·21	26·5
Chromosome 5	*L*	58·90	59·8
	Ic	27·28	28·4

The agreement between the two methods is satisfactory within the limits of the imperfections of the technique but the method of "blurring" has the advantage of bringing out a distinctive difference between the two chromosomes, 4 is longer than 5 (by definition) but also its centromere is a little more distal than that of 5.

Although these are slight differences, this simple observation makes it possible in good quality preparations to make the pairing-off method much more precise.

Group 6–12 and X

Measurement of the chromosomes of this group and plotting each of them on the graph gives the image in Fig. 3.8.

The optical arrangement previously indicated reveals the existence of quite

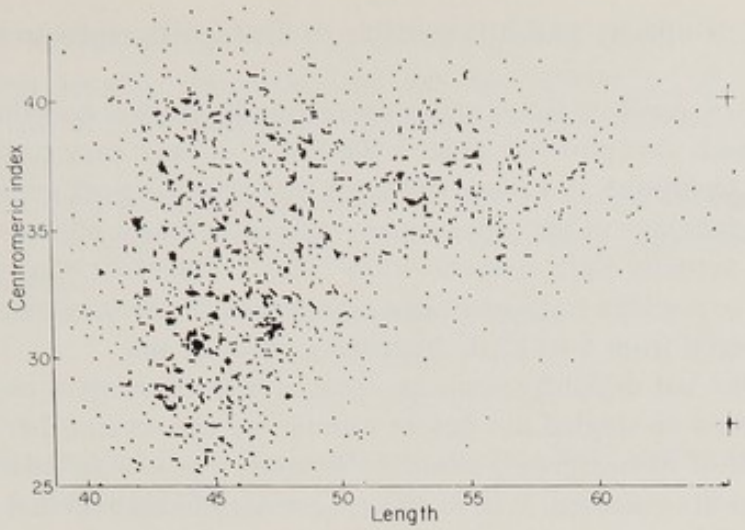


FIG. 3.8

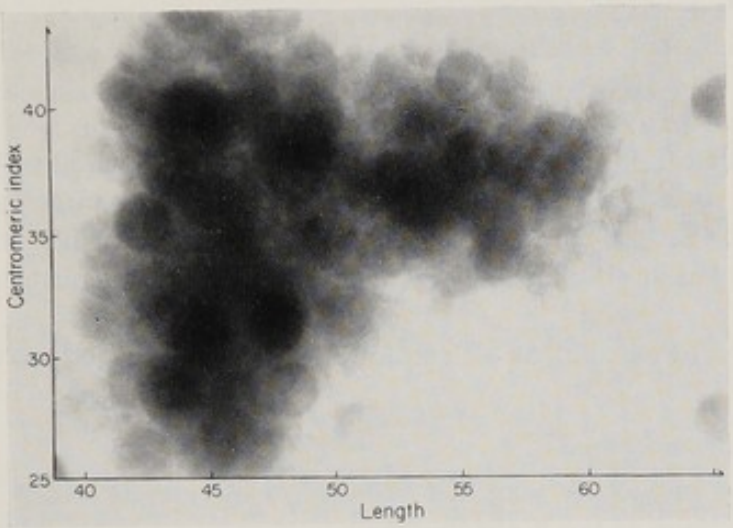


FIG. 3.9

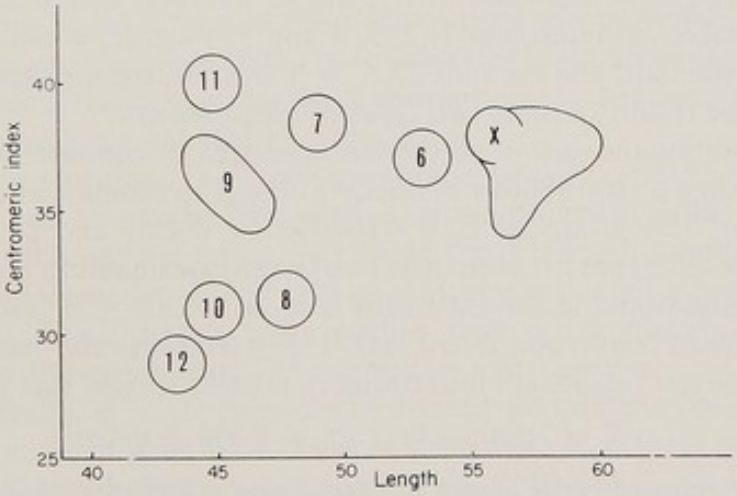


FIG. 3.10

distinct zones of opacity and it is possible to distinguish eight groups or spots (Fig. 3.9).

The centre of each of these spots corresponds to two coordinates which characterize each chromosome (Fig. 3.10) and it is very easy to compare the results of this technique and those obtained by the pairing-off method.

The main advantage of the topographic method is that it allows us to specify that there are actually eight concentration "sites". Therefore it is reasonable to admit that it is possible to characterize eight types of chromosomes which can then be numbered from 6 to 12 in decreasing order of size.

However, one last difficulty remains—that of identification of the X chromosome which must be singled out before numbering the seven other pairs.

All methods of measurement entail an accessory postulate which limits the value of the results obtained. It is, in effect, necessary to assume that the single X chromosome of the male is morphologically identical with the two in the female. This supposition may turn out to be false if heteropycnosis of the X chromosome manifests itself by variations in length at metaphase.

Statistical identification of the X chromosome

From statistical comparison of 50 cells with two X and 50 cells with one X chromosome it should be possible to determine the characteristics of this chromosome. Several methods of analysis are conceivable including that proposed by Patau (1960a), which makes use of quite intricate mathematical formulation.

To simplify the calculation we employ two general methods to determine the length of X.

(a) Method of unknown X

As we have previously seen, the usual term of reference 22 A + X requires prior identification of the X chromosome in the male cell. To obtain a standard independent of this prior identification the relative length of each of the chromosomes 6–12 and X is measured in relation to the total length of all the large easily identifiable chromosomes 1, 2, 3, 4 and 5. Further, a conversion factor between this standard and the sum 22 A + X is estimated for each cell in order to compare the results of these two types of measurement.

Then, by adding the sum of all the medium-sized chromosomes in 50 female cells and dividing by the number of cells, we obtain an estimate of the mean total length (L_1) of this group or $L_1\ ♀ = 106{\cdot}6981 \pm 0{\cdot}4569$ and $L_1\ ♂ = 99{\cdot}1776 \pm 0{\cdot}3779$. The difference between these two lengths ascribable to the absence of a second X chromosome in the male cells is $X_1 = 7{\cdot}5205 \pm 0{\cdot}5930$.

Since the mean conversion factor is $0{\cdot}7173 \pm 0{\cdot}0024$ in the female cells it is possible to convert this length into ordinary relative length, that is:

$$X = 7{\cdot}5205 \times 0{\cdot}71728 = 5{\cdot}394 \pm 0{\cdot}426$$

which finally represents the length of chromosome X in relation to the classical standard (22 A + X).

(b) Method of known X

This rather lengthy demonstration has the advantage of confirming the validity of use of the term 22 A + X even after identification *a priori* of the X chromosome.

In fact, the same method of calculation based on the difference of total length (L_2) of the 16 medium-sized female and the 15 medium-sized male results in the following figures:

$$L_2 \, ♀ = 76{\cdot}6994 \pm 0{\cdot}158$$
$$L_2 \, ♂ = 71{\cdot}2576 \pm 0{\cdot}138$$
$$X_2 = 5{\cdot}442 \pm 0{\cdot}211$$

It will be seen that these two estimations agree in assigning a length of about 5·5 to the X chromosome.

Estimation of the site of the centromere of the X chromosome

A calculation identical with the preceding one consists in summing the centromeric indices of the 16 medium-sized chromosomes of the female cells in order to obtain a mean sum of the centromeric indices. (This latter parameter is assimilated in a weight characteristic of each chromosome.)

Estimation of the mean sum of *Ic* for the 15 medium-sized chromosomes of the male cells then enables us to estimate by difference the *Ic* of the X chromosome without the latter having previously been identified.

We find

$$Ic \, ♀ = 5{\cdot}52992 \pm 0{\cdot}02065$$

$$Ic \, ♂ = 5{\cdot}14300 \pm 0{\cdot}02250$$

whence

$$Ic \, X = 0{\cdot}38692 \pm 0{\cdot}031$$

From this analysis we can thus conclude that the position of the X chromosome in the karyogram in Fig. 3.10 must correspond approximately to the coordinates: $L = 54{\cdot}0$ and $Ic = 0{\cdot}386$.

This point corresponds in Fig. 3.9 to the group furthest to the right, that is, corresponding to the largest of the medium-sized chromosomes.

This technique therefore allows us to subscribe to the consensus of the authors of the Denver classification that the X chromosome is the largest of the medium-sized ones and has a submedian contromere.

Having thus localized the X chromosome it is easy to return to the karyogram (Fig. 3.10) and to number the various chromosomes in order of diminishing size (the position of the centromere being the determining element).

Six is a little smaller than X and its centromere though submedian is a little more distal, 7 is distinctly more median than 6, 8 is frankly distal, 9 is submedian and 10 frankly distal, 11 submedian and 12 distal.

Schematically, the eight pairs can be divided according to the position of their centromeres: three pairs have distal centromeres ($Ic < 0{\cdot}33$) at less than one-third of their length. These are in order of size 8, 10 and 12. The other four have a submedian centromere and essentially differ in size. This distribution into

two groups was the basis of one of the classifications prior to Denver (Lejeune *et al.*, 1959).

The coordinates of the karyogram are as follows:

	L	*Ic*
X	55·0	38·0
6	52·5	36·5
7	48·5	38·5
8	47·5	31·5
9	45·5	35·0
10	44·5	30·5
11	44·0	39·0
12	43·5	28·5

Group 13–14–15

This group presents quite special difficulties because of the nearly terminal position of the centromere. Under these conditions, the precision of measurement of the centromeric index is illusory and the topographic method previously used cannot be applied.

The actual length of the six elements varies little and does not allow us to separate the group into three distinct pairs.

We are left with the satellites (Chapter 3) which frequently accompany these chromosomes. This cytological feature has given rise to much discussion (which will be treated later) and one can summarize, in a few words, the present state of uncertainty.

It is impossible to distinguish formally these three pairs from each other and it is necessary to apply the Denver decision and to give the number 13 to the pair bearing the best formed satellites, 14, the pair which bears small ones and 15, that with none at all (or almost none). It is essential to make it clear that this assignment of a number to each of the pairs is deceptive in the sense that it is by no means certain that the two elements coupled together are really homologues (in the genetic sense of the term) and not simple analogues (in the morphological sense).

Group 16–17–18

The existence of short arms large enough to measure enables us to definitively classify these three pairs by the topographic method.

The photographs in Figs. 3.11, 3.12 and 3.13 reveal the existence of three "sites" the coordinates of which are as follows:

Chromosome	"Blurring" method		Pairing-off method	
	L	*Ic*	*L*	*Ic*
16	29·5	0·41	29·9	0·40
17	29·0	0·32	28·9	0·31
18	25·0	0·27	25·5	0·28

However, it should be noted that since the area of maximum opacity of 17 overlaps that of 18 (Fig. 3.12) these estimates cannot be regarded as absolute. But, the direction of the major axis of this cloud along a bisector of the axis of the coordinates suggests that the larger of the two (17) has a centromere more median than the smallest one (18). Agreement between the topographic and pairing-off method is highly satisfactory as shown by the above table.

Group 19–20

No procedure has so far demonstrated a definite difference between these two pairs. At most, it may be supposed that pair 19 is a little larger than pair 20 but the almost median position of the centromere precludes use of the topographic method.

Group 21–22

Here too, topographic analysis cannot be used. The extreme smallness of the short arm removes any significance from the calculation of a centromeric index and the exclusion of satellites eliminates an essential part of the data.

However, the following conclusions may be drawn from accumulation of many observations:

(a) Size

Chromosome 21 is probably smaller than 22, the retention of this ordinal number is, however, necessary owing to the aetiological role of this chromosome in mongolism. As the term trisomy 21 is now widely adopted, it would appear useless and dangerous to amend this usage.

(b) Position of the centromere

The small chromatic arms of chromosome 21 are extremely short and sometimes not even discernible. The arms of 22, though very small themselves, are usually larger and more easily identifiable.

(c) Presence of satellites

The satellites attached to the short arm by a small filament are usually well-developed on chromosome 21 (cf. Chapter 4). On the other hand, they are usually not found on chromosome 22. However, it is sometimes possible to observe satellites on three and much more rarely, on four of the small acrocentrics. In this case, the satellites of chromosome 21 are always much better developed than those of 22 (Petersen and Therkelsen, 1961).

It is useful to recall here what has been said of group 13–15, namely, that these appendages are not sufficiently constant to justify formal identification (Edwards, 1961b; Ferguson-Smith and Handmaker, 1961; Gromults and Hirschhorn, 1962).

Independently of their very real physiological variance, the satellites present a major difficulty in observation; their visualization depends not only on the cell stage but also on the staining technique. Thus, O. J. Miller and Mukherjee

FIG. 3.13

FIG. 3.12

FIG. 3.11

(1962d) in a systematic study observed that the mean number of satellites per cell was higher for Feulgen than for orcein preparations. In this respect, staining with Unna's blue, advocated in the previous chapter, appears to permit excellent detection of the satellites.

Establishment of the karyotype in practice

The many observations summarized above permit us to propose a simple and rational method for establishing the karyotype. Although the following remarks are more "tricks of the trade" than scientific, they may help beginners to avoid useless groping and time loss.

After cutting up the photograph into individual chromosomes they are systematically arranged in the seven groups and then the homologous chromosomes are paired within the group. It may be possible to use a computer coupled with a television camera to analyse the mitotic plaques automatically, as envisaged by Falek and Neurath (1963). However, analysis of the karyotype described below is likely to remain the method of choice for quite a long time to come.

(a) The 10 large chromosomes

Among these it is easy to recognize chromosome 1 (the largest with median centromere), chromosome 2 a little shorter than 1 and with a more distal centromere and chromosome 3 considerably shorter than 1 but with a similar median centromere.

Pairing off homologous chromosomes for these three types raises no difficulty. The remaining four chromosomes can then be paired two by two, using the following criterion: pair 4 is a little longer and has a more distal centromere than pair 5. Taking these two factors into account the discrimination of pairs 4 and 5 can be accomplished unambiguously in about 50 per cent of "excellent" cells.

(b) The 15 or 16 medium-sized chromosomes.

A priori, classifying these chromosomes would appear to be a very long and highly uncertain venture. Thus, we must make maximum use of the knowledge which may be drawn from karyogram 3.10 and proceed in the following fashion. Select the six medium-sized chromosomes with a distal centromere, that is, situated at least one-third along the length of the element. In "excellent" cells such selection presents no ambiguity.

These six chromosomes are then paired two by two according to their similarity (size and position of centromere), the largest of the three pairs is numbered 8, the second 10 and the third 12.

Then the remaining nine or ten (according to whether it is a male or female) are paired two by two, the determination of the X chromosome being made following the rule that it is the longest in the group (although its size may be very close to 6) and has a centromere a little more median that that of 6. In the male, diagnosis by exclusion (X is the unpaired chromosome) will confirm this choice *a priori*.

Finally, the remaining four pairs are best arranged in order of diminishing size, assigning to them Nos. 6, 7, 9 and 11.

This method, which makes full use of the statistical knowledge previously outlined, helps to establish karyotypes according to a systematic plan, with an error not greater than one rank, inside the two subclasses.

The following table shows more precisely the extent of the remaining uncertainty:

No. assigned by systematic classification	No. which another observer may propose. This uncertainty representing a "maximum"
X	6
6	X or 7
7	6 or 9
8	10
9	7
10	8 or 12
12	10

It seems to us essential to bear in mind this residual uncertainty since although the numerical notation makes it necessary to assign a given number to a given element, it is necessary to know how far this is an observational certainty or a purely categoric inference.

Group 13–15

These six acrocentric medium-sized chromosomes are best paired two by two and numbered according to the following rule—the pair bearing the larger satellites is 13, that with smaller satellites 14 and the third, without or with very small satellites, is 15.

This systematic distribution, as stated above, may not perhaps correspond to biological reality and it should be remembered that formal identification of 13 or 15 is at the limits of existing possibilities.

Group 16–18

Easily separated from the preceding groups these six chromosomes are paired two by two, chromosome 16 being the largest with a paramedian centromere. In pairing off 17 and 18 it is necessary to remember that 18 is smaller then 17 and has a more distal centromere. These two criteria permit 90 per cent unambiguous identification in "excellent" cells.

Group 19–20

These small chromosomes in the form of St. Andrew's crosses are best paired two by two, the largest pair being numbered 19.

There is no formal criterion for differentiating between these two pairs.

Group 21–22–Y

Among the five small V-shaped acrocentrics one should first distinguish (for a male) the Y chromosome. In over half of very good cells the strict parallelism of the two chromatids without constriction at the level of the centromere permits visual identification. This characteristic aspect very probably deriving from early separation of the centromere of the Y chromosome is, however, not constant and the Y then appears identical with the 22 although a little longer. To our knowledge, the normal Y chromosome is never satellited.

In the female, only the four remaining acrocentrics are separated according to the already outlined criteria—the 21 is smaller, has short, almost undetectable arms and is satellited, while the 22 is longer, has short distinct arms and no (or few) satellites.

Systematic observation shows that such a classification is morphologically unambiguous in 30–40 per cent of "excellent" cells. But classification is often impossible when the chromosomes have been too greatly contracted by colchicine or when they have been damaged by too brutal a hypotonic shock.

Features of certain chromosomes

The preceding description of the normal human karyotype would be very incomplete if certain structural features were not mentioned.

Apart from the satellite there may exist zones of secondary constriction or, to express the observed facts more directly, zones of less intense staining of the chromosomal rod.

These much brighter zones may be the result either of constriction of chromatin or of uncoiling of the secondary spiral.

Two cases are particularly worthy of note, that of chromosome 1 and that of the medium-sized chromosomes.

Chromosome 1

The existence of a zone of reduced opacity at a very small distance from the centromere has been pointed out by many workers (Patau *et al.*, 1961 a) and we ourselves have frequently observed it. This structural detail unfortunately is of little interest since it adds nothing to the precision with which we can identify chromosome 1, the most easily identified in all the karyotype.

Medium-sized chromosomes

A secondary constriction in one or more medium-sized chromosomes has been repeatedly observed [de la Chapelle, 1961 a; Muldal and Ockey, 1961; Dobson and Ohnuki, 1961; Patau *et al.*, 1961 d). Recently, quite harsh treatments were proposed to bring out these poorly staining zones which may often be considered as zones of stretching prior to rupture (Saksela and Moorhead, 1962; Sasaki and Makino, 1963). These methods give relatively comparable results and one is

even tempted to draw a parallel between the results observed and the observations with tritiated thymidine labelling (Schmid, 1963).

These investigations are far from complete and it seems prudent to conclude that at the present time the secondary constrictions are phenomena of observation which cannot be considered as stable morphological features for identification of a particular chromosome. It should be added that these heterochromatic regions are much more easy to recognize in mitoses in blood cells than in fibroblasts where they are exceptional.

Identification of abnormal chromosomes

Existence of anomalies of the human karyotype, the focus of interest in human cytogenetics, raises a difficult problem of identification and denomination.

The study of the various known anomalies will be undertaken in the following chapters but it is essential to examine this problem straight away from the angle of classification.

The absence of a chromosome or its presence in excess does not raise special morphological difficulties since identification by absence or excess is based on the previously adopted criteria.

On the other hand, structural rearrangements raise a problem first of detection and then of interpretation.

To simplify, we may consider two general types of rearrangement, those which entail a loss or gain in chromosome material and those which correspond to a rearrangement in structure without appreciable change in quantity.

(a) Loss or gain of chromosome material

Deletions, duplications and translocations correspond to these different types.

From the purely morphological viewpoint it is essential to establish the limits of detection of these accidents.

The uncertainty concerning the length of a chromosome may be as a first approximation expressed by the standard deviation in distribution of the relative lengths observed in a large number of homologous chromosomes. In this connection, Table 3.4 reminds us that chromosome 1 may "normally" vary between 96·8 and 79·4 without our being able to consider that 96·8 is too great or 79·4 too small. In absolute terms in order to affirm that a chromosome has undergone a loss or increase in length it is neccessary that the difference between observation and the mean theoretical value is of the order of 10 per cent of the length of the element itself for large or medium chromosomes and of the order of 15–20 per cent for small ones.

Another approach consists in comparing the two homologues of a pair recognized with certainty and in relating the difference in their length to the mean length of the pair. This calculation for chromosome 1 in 48 male cells gives a mean difference of 5·8 $\pm$ 4·4 per cent and for chromosome 2 the same calculation gives 5·7 $\pm$ 5·2 per cent.

It is therefore evident that even systematic comparison within pairs does not appreciably increase the accuracy of the measurements and it is reasonable to conclude that a loss or gain in substance in a medium-sized or large chromosome

must be of the order of 10 per cent of the length of the element in order to be detected with certainty. This reflection also explains the difficulties of classification within the groups discussed previously.

However, it must be pointed out that a deletion or a duplication affecting only one chromosome arm has the effect of not only changing the total length of the element but also the centromeric index (since one of the arms remains unchanged).

Here, the accuracy of the identification becomes obviously greater since for chromosome 1, for example, the centromeric index is $46{\cdot}47 \pm 2{\cdot}14$. That is to say, an index less than 43 would be recognized as abnormal, which would involve only a 6 per cent reduction in the short arm.

The same observation applies to chromosomes 2, 3, 4 and 5. However, if we are dealing with one of the medium ones, a gain or loss of substance in one of them may from the start systematically falsify the classification which would then render futile any determination. On the other hand, deletion of the short arm of chromosome 18 is perfectly detectable (Grouchy *et al.*, 1963j) as is part of the short arm of 21 or 22 (Shaw, 1962) or the loss of half the short arm of chromosome 5 (Lejeune *et al.*, 1963b).

From this discussion we may, in general, conclude that the centromeric index is more accurate in detection of a change in length than the relative length of the element itself. In practice, we consider that for a variation in length to be detected with certainty it must be at least 10 per cent of the element and that change in the centromeric index must also approach 10 per cent of the value of the index to be significant.

These few reflections, though a little tedious, will prove necessary for an understanding of the very important problem of possible partial trisomies and masked translocations.

(b) Translocations

These changes, which nearly always consist of rearrangement between two acrocentric chromosomes, present hardly any difficulty of detection. In fact, the centromeric fusion of two acrocentrics produces in their place a large mediocentric chromosome (fusion of two large acrocentrics), a submedian medium-sized one (fusion of large and small acrocentrics) or a small mediocentric (fusion of two small acrocentrics).

In all cases the existence of the new chromosome involves a morphological change too obvious to be overlooked. On the other hand, the identity of the two elements implicated in the translocation can only be established by elimination (the elements are missing) or by genetic inference.

It must be stressed that reciprocal translocations of fragments of the same dimensions cannot be detected if these changes involve no change in the centromeric index of the affected chromosomes.

Designation of abnormal chromosomes

The problem of nomenclature of abnormal chromosomes dealt with in the Denver document is still not satisfactorily resolved. The proposal to designate

the new element by the initials of the town in which it was discovered followed by a number indicating the order of the successive discoveries has so far been applied only to the amputated acrocentric encountered in chronic granulocytic leukaemias called Ph^1 (cf. p. 145).

The major difficulty with this approach is that the anomaly described in a case of Turner's syndrome with a translocation $2 \sim 22$ ought to be called Pa^5 which does little to enlighten the reader.

Within the limits of existing possibilities it therefore seems to us desirable to describe translocations by indicating:

(a) the morphology of the new element;
(b) the chromosomes involved when this is possible (ex. translocation $2 \sim 22$);
(c) the group of rearranged chromosomes, for example, ex. translocation $(13–15) \sim (21–22)$ or again a $D \sim G$ translocation.

CHAPTER 4

Trisomy 21

BACKGROUND AND DEFINITIONS

The curious mental disease which was later to be improperly termed "mongolism" was noticed for the first time by Esquirol (1838). This alienist enlisted "to serve the history of idiocy" those subjects whose stature is short, the head not very voluminous, the external palpebral commissure higher than the internal one, the nose depressed at its root.

F. Seguin (1846) added to this description the truncated nose, the thick and furrowed tongue and the sensitivity of lungs and integuments to infections. In 1866 he spoke of *diathése furfuracée* of these "*bons enfants*" who can learn to speak and acquire some knowledge.

Langdon Down in 1866 very rightly laid emphasis on the stereotyped physionomy and behaviour of these patients. "This is so marked that placed side by side it is difficult to believe that the specimens thus compared are not the children of the same parents", but carried away by his desire to class idiocies as a function of ethnic appearances, he described this backwardness as "mongolian idiocy" and placed it after "negroid" and "Malaysian" idiocy.

This inappropriate name was the sole survivor in the foreseeable collapse of such a classification. In fact, this individualization by pseudo-mongolian facies, which still remains one of the major elements in its diagnosis, has given wide currency to the term mongolism despite the very regrettable confusion it entails.

Adoption of this "erroneous" term is all the more regrettable since Seguin (1846 and 1866) described this disease before the publication of Langdon Down under the name "furfuraceous idiocy" with a precision which Benda (1962) considers the "most ingenious description of physical characteristics of the mongoloid growth deficiency"—"furfuraceous cretinism with its milk white rosy and peeling skin; with its shortcoming of all the integuments, which give an unfinished aspect to the truncated fingers and nose; with its cracked lips and tongue, with its red, ectopic conjunctiva, coming out to supply the curtailed skin at the margin of the lids" (Seguin: *Idiocy and its Treatment by the Physiological Method*).

This brief recapitulation of the discovery of the clinical entity calls for particular caution in using a new term as was recently proposed (Allen *et al.*, 1961) to replace the the improper term mongolism.

The term Down's syndrome, rejected by some (Spalding, 1961), can hardly be supported, since it would compound a double error, historical and aetiological; historical, since Seguin was the first to describe the disorder, aetiological

since the disease has absolutely no connection with the racial hypothesis of Langdon Down.

As we shall subsequently see, the widely established uniqueness of the chromosome aberration responsible for the disorder enables us to name it after its now known cause. The term "mongolism" will be deliberately replaced in this book by trisomy 21.

1. CLINICAL AND EPIDEMIOLOGICAL ASPECTS

The typical facies of children and especially of very young children permits a very early clinical diagnosis usually right from birth and the "mask" cast by the disorder on the child's own characteristics produces such a resemblance between these small patients that after seeing one of them there is no risk of mistaking them (Turpin, 1931). Cranial features even permit a retrospective diagnosis in archeological specimens (Brothwell, 1960).

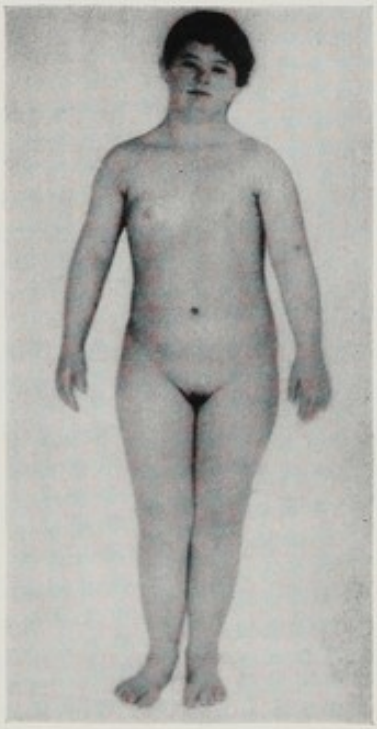


FIG. 4.1.
Frontal view of trisomic 21 female.

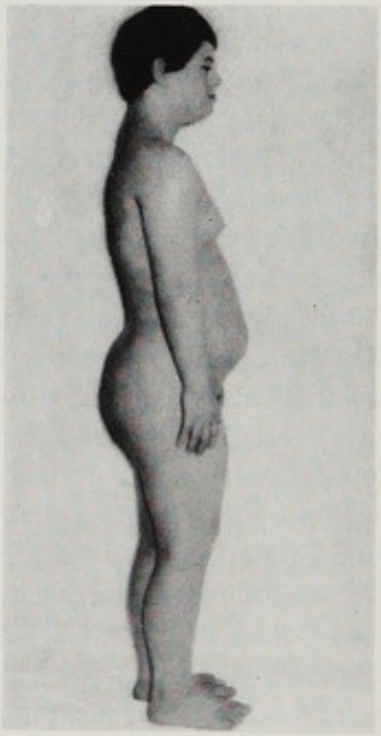


FIG. 4.2.
Side view of trisomic 21 female.

The clinical description of the disturbances both morphological—from shortness to epicanthus—and functional—from hypotonia to psychomotor retardation and eye (Eissler and Longenecker, 1962) bone (Rodighiero and Scapinelli, 1962) and heart (Rowe and Uchida, 1961; Rowe, 1962) malformations—has been given so many times and with such precision that we shall refer the reader to earlier general reviews (Øster, 1953; Lejeune, 1960; Benda, 1960).

We merely note for the sake of the record that the incidence of heart defects put at 40 per cent by Rowe and Uchida (1961) and at 56 per cent by Berg *et al.* (1960) appears in the last analysis to be a little lower in trisomy 21 than in the two other trisomies (13 and 18).

According to the statistics of Rowe and Uchida (1961) and Nouaille and Gautier (1963), the distribution of the different types of malformation seems quite characteristic. A common atrio-ventricular canal comes first (36–47 per cent of the cases) followed by interventricular communication (26–33 per cent), then patent ductus arteriosus (8–10 per cent), with various other types making up the remainder; ostium secundum, aberrent subclavicular artery, tetralogy of Fallot, simple pulmonary stenosis, multiple shunts, etc.

The mental debility of subjects with trisomy 21 though often studied has so far revealed no symptomatic peculiarities apart from limitation of the faculties of abstraction, which prohibits an IQ higher than 70 (Dundson, 1960) and progressive deterioration with age (Zeaman and House, 1962). The existence of subjects with trisomy 21 of normal intelligence as postulated by certain authors (Webster, 1963) has never been demonstrated apart from cases of mosaicism (cf. below).

We shall mention in greater detail certain characteristics of the disorder to which the discovery of trisomy 21 lends more immediate theoretical interest.

For greater simplicity these features may be grouped into three categories each of which reveals one of the aspects of the disorder: its constitutional nature, its genetic determinism and, finally, its mode of appearance.

(a) Constitutional nature

Right from birth, hypotonia coupled with the characteristic morphology of trisomy 21 permits a usually unequivocal diagnosis.

One of the most characteristic morphological traits is the anomaly of the epidermal structures of the fingers, the palms of the hands and the soles of the feet.

Dermatoglyphics

The sinuous elevations of the epidermis which form the palmar and plantar ridges give concentric structures which form various patterns: loops, arches, whorls and triradii. These are visible from birth and remain unchanged throughout the development of the individual. On these basic structures are superimposed the flexion creases of the fingers and palms which are also perfectly stable (Fig. 4.3). These patterns are laid down before the first month *in utero* (Turpin *et al.*, 1955) and their anomalies demonstrate the existence of exceptionally early disturbance in embryogenesis. The pointed unfinished aspect of the ridges of the hypothenar eminence is very typical of infants less than a year old with trisomy 21 (Wolf *et al.*, 1963).

Finally, the influence of the genetic constitution of the individual on these patterns has been amply demonstrated (Holt, 1961).

The dermatoglyphics in trisomy 21 have been the subject of widespread study

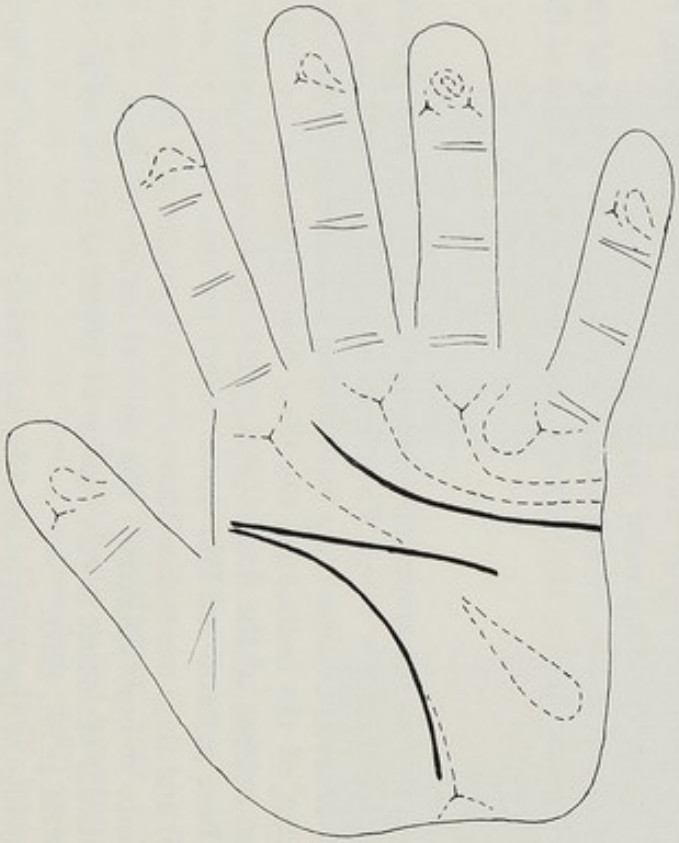


FIG. 4.3. Right hand of normal subject (diagrammatic). Note (1) presence of three normal flexion creases; (2) axial triradius (position t); (3) radial loop on hypothenar eminence, a formation never found in trisomic 21 subjects; (4) oblique direction of the ridges of the distal part of the palm and presence of a pattern between the 4th and 5th fingers; (5) digital patterns: I, cubital loop; II, arch; III, cubital loop; IV, whorl; V, cubital loop.

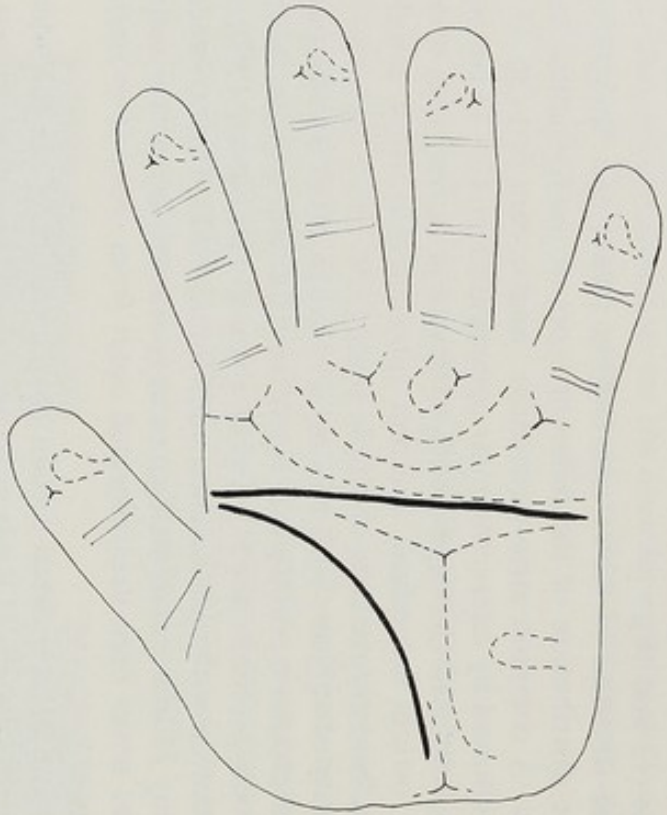


FIG. 4.4. Right hand of a subject with trisomy 21 (diagrammatic). Note: (1) transverse palmary crease; (2) transverse direction of the ridges of the distal part of the hand; (3) pattern between 3rd and 4th fingers; (4) medio-palmar axial triradius (position t″); (5) loop with cubital opening on the hypothenar eminence; (6) single flexion crease on 5th finger; (7) loop with radial opening on the 4th finger.

(Cummins, 1936; Penrose, 1954c; Lejeune, 1955) and without going into a detailed description, it is possible to establish the diagnosis from the following four criteria (Fig. 4.4): transverse orientation of the papillary ridges of the distal part of the hand instead of the normal oblique orientation; presence of a loop with an ulnar opening on the hypothenar eminence; elevation of the axial triradius to a mediopalmar position; and presence of only a single flexion crease of the hand (transverse palmar crease) resulting from coalescence of the two normal flexion creases popularly known as head line and heart line. A statistical study shows (Turpin and Lejeune, 1953a) that a print classification by these four categories permits diagnosis of trisomy 21 in 95 per cent of the cases.

As Crookshank (1931) had already pointed out, the transverse crease rare in the normal human (1 per cent) and present in 68 per cent of subjects with trisomy 21 is a taxonomic feature typical of lower simians.

Comparative study of simian dermatoglyphics (Turpin and Lejeune, 1954a, b) shows that the above four criteria are specific characters of lower simians and not of anthropoids (orang-utang, gorilla, chimpanzee). Trisomy 21 thus reproduces in man very primitive specific characteristics.

On the finger tips, the numerous epidermal ridges making up a fingerprint (Holt, 1961) have a special distribution in subjects with trisomy 21. Other features are known: the abnormal frequency of loops with radial openings on the fourth finger (Penrose, 1961), the narrowing of the distance between the two flexion creases of the little finger or the existence of a single crease at this level which emphasizes the brachymesophalangy. This feature may also be transmitted as a dominant trait apart from any trisomy 21 (Lejeune *et al.*, 1958). These various features suggest that some of the signs responsible for these dermatoglyphic characters must be situated on chromosome 21 (Lejeune, 1960). However, the discovery of different dermatoglyphic anomalies, as in the trisomies 13 and 18, by Uchida *et al.* (1962c) confirmed by Penrose (1963a) and also in the syndrome of deletion of the short arm of chromosome 5 (Lejeune *et al.*, 1963b) suggests that there are many independent genes whose harmonious balance determines the dermatoglyphic patterns of the normal individual. It has also been suggested (Gobesso and Piazzi, 1962) that the advanced age of the mother may accentuate these features in the affected child.

Index of nuclear segmentation of the granulocytes

The first cellular anomaly linked to the disorder was described in 1947 by Turpin and Bernyer (Fig. 4.5). It is known that segmentation of the nucleus of the polynuclear leukocytes may be more or less pronounced giving figures with 1, 2, 3, 4 and even 5 or more lobules. The frequency distribution of these different types clearly deviates towards types with one or two lobules in subjects with trisomy 21. The numerous confirmations of this fact suggests that here we are dealing with very constant nuclear stigmata independent of any reaction to an infectious process (Shapiro, 1949; Lüers and Lüers, 1954; Mittwoch, 1957; Caevini and Maderna, 1962). The small nuclear appendage known as the "drumstick" typical of the female sex is also less frequently found in girls with trisomy 21 than in normal females (Mittwoch, 1961).

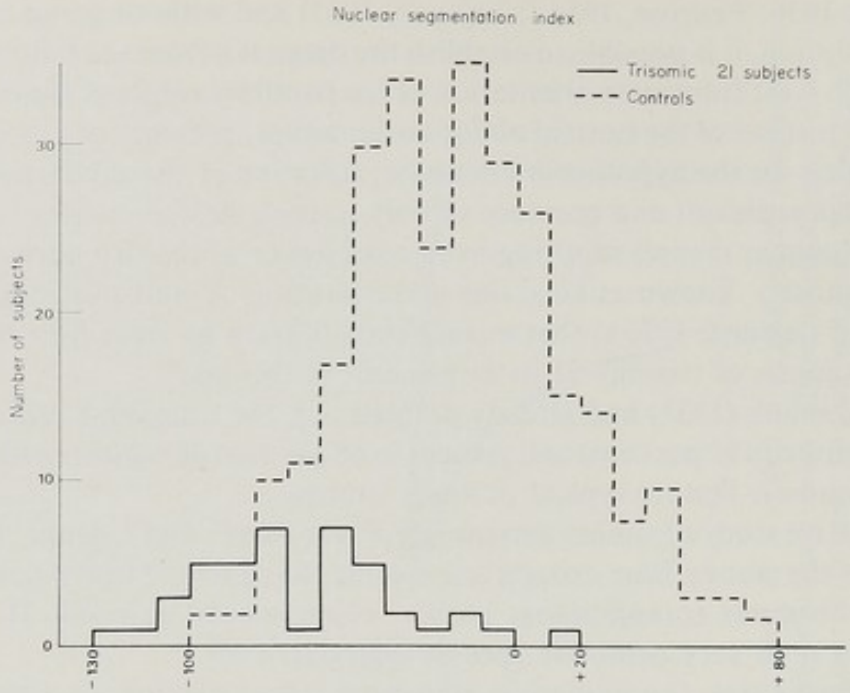


FIG. 4.5. Nuclear segmentation of polynuclear cells. It will be seen that in trisomy 21 there is a tendency for the nucleus to have a small number of lobules. Shift to the left in Arneth's formula. (After Turpin and Bernyer, 1947.)

Sensitivity to leukaemic process

The special incidence of acute leukaemias in subjects with trisomy 21 was recognized even before the chromosomal nature of the disorder was demonstrated (Schunk and Lehman, 1954; Bernard *et al.*, 1955; Krivit and Good, 1956; Merrit and Harris, 1956; Penrose, 1956; A. Stewart *et al.*, 1958; A. Stewart and Hewitt, 1959). The most recent statistics (A. Stewart, 1961; Wald *et al.*, 1961; Holland *et al.*, 1962) show that acute leukaemia is almost twenty times as frequent in trisomy 21 as in normal subjects. The biological implications of this association will be specifically discussed in Chapter 8.

(b) Genetic determinism of the disorder

Observations on twins

Comparison of monozygotic and dizygotic twins is a simple method for demonstrating the genetic component of a congenital disorder.

Table 4.1, below, presents the few twin pregnancies (158) reported in the literature and permits us to state that no case of certain dizygotic twins (different sexes) both with trisomy 21 has been reported and that the 13 certain monozygotes are all concordant.

It should be noted that the three cases of concordant dizygotes (Russell, 1933; McKay, 1936; Jervis, 1943) may spring either from an error in the diagnosis of twinning or a random association.

The concordance between monozygotic twins indicates that the determinant lesion must be an early one (before the 15th day *in utero*) and thus derive from a genetic mechanism. The much more recent discovery of discordant monozygotic twins for trisomy 21, heterokaryotic monozygotism (Lejeune *et al.*, 1962d; de Wolff, 1962), allows us in this last case to state the precise stage of appearance of the lesion—at cleavage of the first blastomeres (cf. Chapter 13). It is possible that the unfortunately incomplete observation of Beukering and Vervoorn (1956) corresponds to this type of twinning.

TABLE 4.1

Combined statistics of Øster (1953), Allen and Baroff (1955) and Carter and Evans (1961)

	Only one trisomic 21	Both trisomic 21	Number of pairs
Dizygotes (of different sex)	59	0	59
Monozygotes (same sex, concordant identity tests)	0	13	13
Probable dizygotes but of same sex	36	3	39
Debatable zygoty but of same sex	33	14	47
	128	30	158

Children born to mothers with trisomy 21

The very rare cases of reproduction in a female with trisomy 21 are presented in Table 4.2. Only observations providing sufficient grounds for firm establishment of the diagnosis have been tabulated. For example, the two normal children reported by Emmet-Holt (1918) and quoted by Hanhart (1960) have been excluded. Likewise, the child described separately by Rehn and Thomas (1957) and by Stiles (1958) is referred exclusively to the authors of the first publication.

In all, of thirteen pregnancies (including one pair of twins) in eleven mothers with trisomy 21, we find five children themselves with trisomy 21 (two confirmed cytologically), seven normal children (including a monozygotic pair), a female, macerated foetus and one abnormal girl certainly not trisomic for chromosome 21.

This distribution, five subjects with trisomy 21, against seven nontrisomics (if we count the monozygotic pair as a single pregnancy) does not differ from the 1:1 ratio expected for the disorder if resulting from a single dominant genetic event.

Moreover, as already pointed out by Penrose (1961), the trisomic 21 mothers were relatively young (mean age 22·17 ± 1·2 years) while their own mothers were much older as is the rule in this disorder (mean age 36·5 ± 3·5) (see below).

Familial recurrence of the disorder

It is classically stated that trisomy 21 singles out one member of an apparently healthy family and does not strike twice. However, the existence of families, with

TABLE 4.2

Children born to trisomic 21 mothers

Source	Age of the mother	Age of the maternal grandmother	Father of the child	Children
Sawyer, 1949 and 1957	25	?	Father of the mother (incest)	Normal girl. At present student of 18 years
Lelong *et al.*, 1949	30	42	Mental defective, not trisomic 21	Boy, clinical trisomy 21, died at 1 month
Rehn and Thomas, 1957 Stiles and Goodman, 1961	19 (tris. 21)	19†	Not known	Trisomic 21 girl
Forssman and Thyssel, 1957 Lehman and Forssman, 1960	20 (tris. 21)	42	Blind epileptic (46 chromosomes N)	Normal boy (46 chromosomes N)
Schlaug, 1957	29	39	Father of the mother (incest)	Abnormal girl. Picture differing markedly from classical trisomy 21
Hanhart, 1960	21 (tris. 21)	44	Brother of the mother (incest)	Girl, trisomic 21
Hanhart *et al.*, 1961	23		Mental defective (46 chromosomes N)	Boy, typical trisomic 21 syndrome, death at $1\frac{1}{2}$ years
Mullins *et al.*, 1960	22	22	Mental defective	Normal boy
Levan and Hsu, 1960	?	?	Not known	Normal boy (46 chromosomes N)
Thuline and Priest, 1961	14 (tris. 21)	?	Not known	Monozygotic male twins, stillborn; 46 normal chromosomes
Thompson, 1961	21	?	Mental defective	Normal boy of 10 years
	20	?	Not known	Female macerated foetus
	22	?	Not known	Stillborn male foetus Probable trisomic 21

† Corrected by Johnson, A.W. and Jaslow, R.I. (1963) Children of mothers with Down's syndrome, *New Engl. J. Med.* **269**: 439–43.

two, three and even five victims (Turpin and Lejeune, 1953a) suggests that certain exceptional cases may be governed by a transmissible mechanism.

However, the precise statistics (Penrose, 1934; Øster, 1953, 1956), though they reveal a slight excess of affected sibs in relation to the number expected (in the event of simple familial coincidence), do not permit statistical confirmation of the reality of this phenomenon. In fact, the influence of maternal age makes statistical comparison difficult, since any child born after a trisomic 21 is by necessity born to an older mother.

The recent statistics of Carter and Evans (1961b) dealing with 642 families with a total of 651 members with trisomy 21 out of a total of 1890 children together with the preceding inquiry by Øster (1956) affords confirmation of this fact. Carter and Evans, taking into account the age of the mothers at the birth of the children, showed that among the sibs born after the birth of the index patients, there were five trisomics 21 while only one was expected and that among the 927 sibs born before the index patients, there were four instead of the 1·5 expected.

In addition, Hamerton *et al.* (1961a) have shown with the same data that the recurrence of the disorder is more probable if the index patient is born to a younger mother.

This familial excess may be largely related to cases of trisomy 21 by translocation (see Chapter 7). However, as the table below reveals, even after exclusion of trisomies by translocation we see that when the mother has had her first trisomic 21 child before 35 years, there is a higher than expected incidence of trisomics among the subsequent children (4 cases against a little less than 1 expected). Such a difference cannot be considered significant but raises the problem of possible familial predisposition to chromosome aberrations or even the existence of gonadal mosaics. In fact, the observation of two trisomics 21 without translocation in the same family is far from being exceptional (Lubs, 1961; Institut de Progénèse, Nos. 318, 804, 895 and 945).

Maternal age at birth of index patient with trisomy 21

	15–24	25–34	35 +	Total
Total number of children	130	341	768	1239
Trisomy 21 by translocation	3	0	0	3
Free trisomy 21	1	3	2	6
Number expected with trisomy 21	0·068	0·606	1·741	2·415

Siblings of index patients in relation to maternal age at the birth of the index patient (after Hamerton et al., *1961)*

Already in 1951, Penrose pointed out that maternal age does not seem to operate in families with trisomy 21 in first cousins. The sisters, both mothers of trisomics 21, showed themselves to be significantly younger at the birth of their abnormal children than the usual age distribution for mothers of trisomic 21 offspring. This effect, discussed by Smith (1960) but verified on a family scale by

the findings previously quoted by Carter *et al.* (1961 b) and Hamerton *et al.* (1961 a, b), shows the rare but certain influence of a familial mechanism of which translocations explain only a part.

Familial accumulation of certain stigmata

Various morphological criteria for the clinical diagnosis of trisomy 21 (epicanthus, transverse palmar crease, furrowed tongue, structure of palmar and plantar prints, etc.) are known to be dependent on Mendelian genes, some recessive, others dominant, and the genetic transmission of these traits in families though perfectly healthy and free of trisomy 21 is a common observation. Existence of a slight excess of these stigmata in families tainted with trisomy 21 has been reported several times; furrowed tongue and transverse palmar crease (Turpin and Caratzali, 1933; Turpin *et al.*, 1947 b), relatively high position of the axial triradius in mothers of children with trisomy 21 and in their sibs but not in their fathers (Penrose, 1954 c).

All these familial tendencies led one to suspect a polygenic mechanism for the disorder in contradiction with the apparently dominant mechanism observed in progeny of trisomic 21 mothers.

(c) Conditions for the appearance of the disease

(a) General incidence in the population

Trisomy 21 is very probably the most frequent of the nosologically defined congenital malformations. In different populations permitting a correct statistical study the incidence of the disease is established between 1 in 600 and 1 in 700 as shown by the following data.

Authors	Region	Frequency
Jenkins (1933)	Chicago	1 in 636
Malpas (1937)	Liverpool	1 in 776
Carter and MacCarthy (1951)	London	1 in 666
Øster (1953)	Zealand	1 in 765
Collman and Stoller (1961)	Australia	1 in 688
Schull and Neel (1962)	Hiroshima and Nagasaki	1 in 785
Wagner (1962)	Honolulu	1 in 478
Jaworska (1962)	Poland	1 in 575

The incidence of trisomy 21 in coloured races has for a long time been considered as lower than in the white race. However, according to the findings of Schull and Neel (1962) in Japan and Wagner (1962) in Honolulu, the incidence of the disease is quite comparable with that of populations of European origin.

(b) Effect of maternal age

As early as 1895, Shuttelworth observed that nearly half the subjects affected belonged to a large family of which they were the last born. In 1909 he demonstrated that mothers of affected children were much older at the birth of the abnormal children than mothers of normal children usually are. Considering that this factor must be of major aetiological importance he described the abnormal subjects as "exhaustion products" resulting from factors unfavourably influencing the reproductive powers of the mother. Jenkins (1933) and Penrose (1933) then showed that the age of the father does not seem to have any influence as such and the possible effect of birth rank was then eliminated by Penrose (1934) although Smith and Record (1955) considered that primogeniture may play a significant role. A possible effect of the age of the father has been discussed in the exceptional cases of the transmission of a 21 ~ 21 translocation (Penrose, 1962a).

The graph (Fig. 4.6) from the data of Penrose (1961) clearly brings out the advanced age of the mothers at the birth of trisomic 21 children. The possibility of a particular effect of the extreme youth of the mothers as postulated by certain workers (Haldane, 1951) seems to be explained by cases of familial transmission of a translocation.

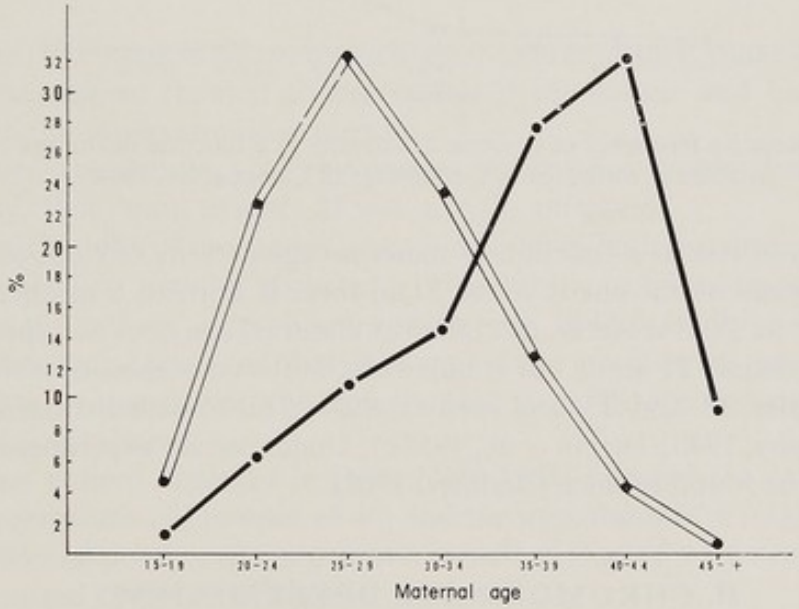


FIG. 4.6. Distribution of maternal age at birth of normal (double line) and trisomic 21 infants (bold line), after Penrose, 1961.

From these curves and taking into account the overall incidence of the disorder it is possible to establish the risk of appearance of a trisomic 21 as a function of the age of the mother at the birth. The recent estimates of Carter *et al.* (1961) are in full agreement with others (Penrose, 1961a; Benda, 1960) and it may be estimated that before the age of 30 the risk is of the order of 1 in 2000. After

the age of 35 it rises to 4 in 1000 to reach a value of the order of 2 per cent after the age of 45. A direct inquiry by Cohen and Warland quoted by Penrose (1961a) into children born in London to mothers of over 45 years revealed the existence of 10 cases of trisomy 21 in the 543 births examined.

Frequency of trisomy 21 in relation to the age of the mother (per 1000 births)

Age of the mother (in years)						
15–19	20–24	25–29	30–34	35–39	40–44	45 +
0·54	0·63	0·74	1·23	3·82	10·72	18·63

After Carter et al., *1961. Figures used as basis for the graph in Fig. 4.7.*

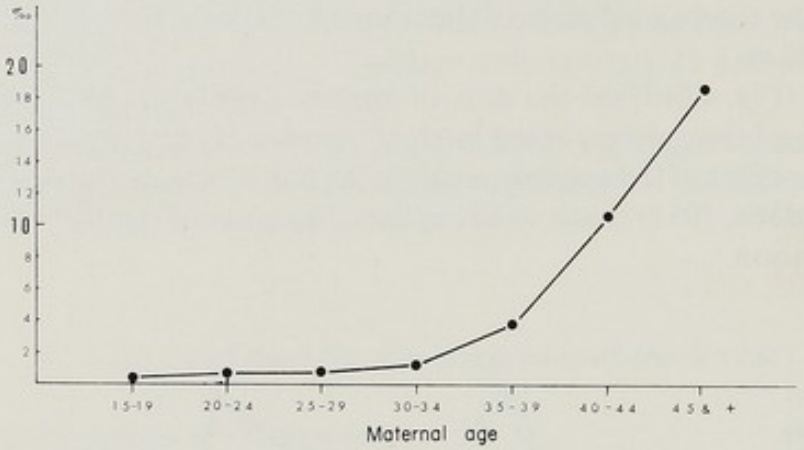


FIG. 4.7. Relative frequency of trisomic 21 subjects as a function of the age of the mother at birth. From the findings of Carter *et al.*, 1961.

Evaluation of risk as a function of maternal age permits obvious progenesic conclusions since about one trisomic 21 in three is born to a mother over 40 years old. As we shall see later, this effect of maternal age does not appear to be confined to trisomy 21 alone but is important in the two others now well established trisomies, 18 and 13, and even extends to all so-called congenital anomalies (Murphy, 1940; Turpin *et al.*, 1953c). Under certain experimental conditions it may be found in mice (Bodmer, 1961).

II. CHROMOSOMAL DETERMINISM

From all the facts reported above it emerges that the disease is a constitutional disorder (specific signs, twin births) which may be transmitted in a dominant mode (reproduction of affected mothers), involving a very large number of genes (familial accumulation of stigmata and Mendelian determinism), that its frequency is very high, ten times greater than other mutations known in man, that no Mendelian hypothesis (dominant or recessive gene) can account for these characters, and that its appearance is influenced by a non-genetic factor (maternal age).

These apparent contradictions, polygenic determinism and dominant transmission, genetic disorder and influence of maternal age can only be reconciled if one postulates a single change affecting a wide segment of the hereditary patrimony, that is, the existence of a chromosome aberration.

The hypothesis of a chromosome mutation responsible for the disorder has several times been suggested.

Thus, Waardenburg in 1932 urged cytologists to see whether or not there exists in this disease some chromosome aberration: "Man sollte einmal beim Mongolismus untersuchen, ob hier vielleicht 'chromosomal deficiency' 'chromosomal duplication' vorliegt. Es ist natürlich auch denkbar, daß nur eine Störung von Chromosomenteilen (Chromomeren): eine 'sectional deficiency' durch 'translocation' oder umgekehrt eine 'sectional duplication' vorliegt."

Bleyer (1934) taking the example of *Oenothera lamarkiana* and *Oenothera lata* postulated a trisomy, a degressive mutation as understood by Hugo de Vries. Leaving to the cytologist the task of verifying this hypothesis, he enumerated the possible figures of 49, 47, 50 and 46 chromosomes.

Turpin and Caratzali in 1937 considering the various aetiological conceptions of this disease considered the hypothesis of "a chromosome anomaly". They gave as an example the bar mutation in *Drosophila* first explained by an inversion then by repetition with a positional effect. Numerous authors now accept that the heterozygous $B/B+$ female is an example of partial trisomy for the bar segment.

Penrose (1939) suggested the hypothesis of a chromosome mutation to account for the exceptional familial concentration of the disease and Fanconi (1939) to explain the observational evidence.

However, the aetiology of the disease remained a keenly debated enigma (Warkany, 1960) until trisomy 21 was actually observed.

Systematic study of the chromosomes of children with this disease was undertaken for the first time by Mittwoch (1952) but the technical imperfections did not allow this author to reach any conclusions. In fact, studying one testicular biopsy Mittwoch concluded that 24 chromosome masses were present and since the number for the species was then thought to be 48, she deduced that it was a normal complement.

The first patient observed in Paris (July 1958) revealed the existence of an extra chromosome (47 instead of 46) and the hypothesis of a fragment by rupture at a special point of chromosome 4 as well as that of a true supernumerary were discussed (Lejeune, 1958a).

In January 1959 study of two other cases provided evidence of the existence of the supernumerary (Lejeune, Gautier and Turpin, 1959e) which was confirmed in February (Lejeune, Gautier and Turpin, 1959c) in a total of 9 patients, and the hypothesis of a trisomy was proposed (Lejeune, Turpin and Gautier, 1959g).

Confirmation of these findings was rapidly supplied by C. E. Ford *et al.* (1959) in a case of trisomy 21 with Klinefelter's syndrome, by Jacobs *et al.* (1959a) in 6 cases of trisomy 21, then by Böök *et al.* in 1959 in 3 cases.

At present the existence of a trisomy for a small acrocentric has been re-

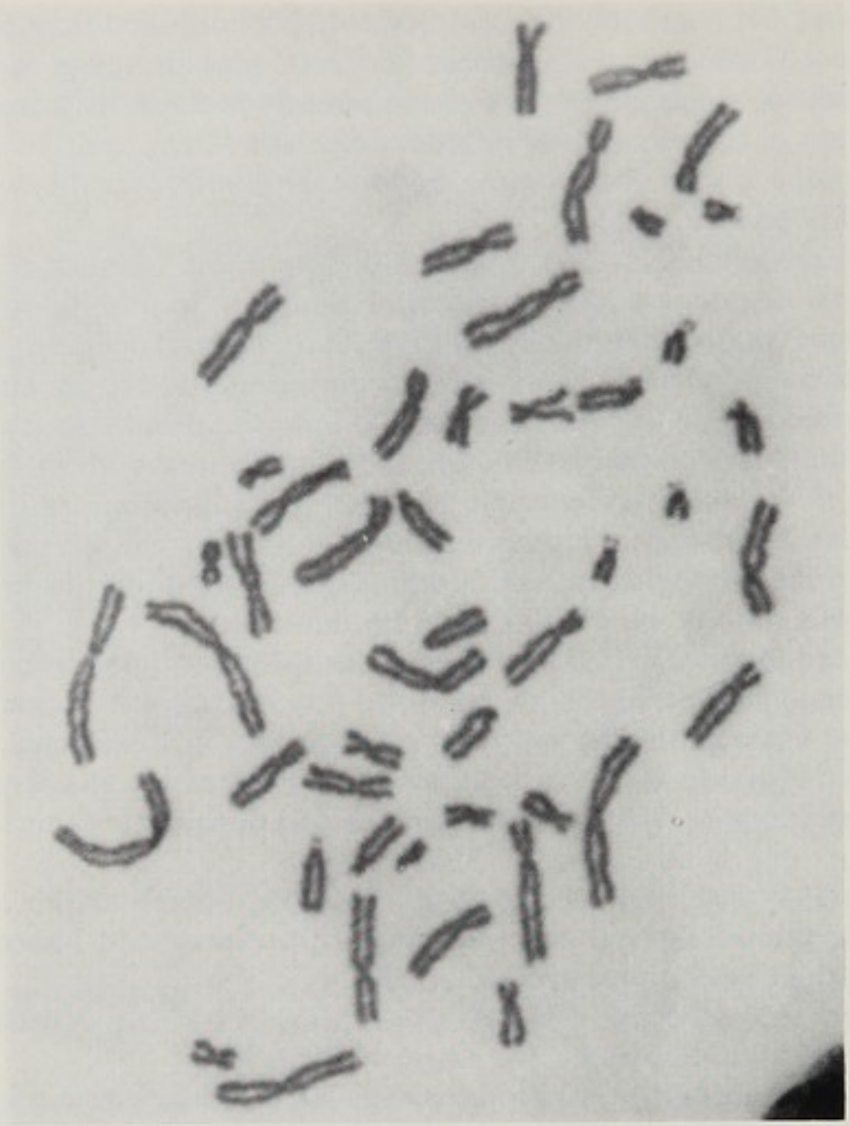


FIG. 4.8. The 47 chromosomes of a trisomic 21 boy (Institut de Progénèse, No. 383).

cognized in all cytogenetic laboratories and in subjects of all races (Makino *et al.*, 1960a, 1962; Lee *et al.*, 1961; Kleisner, 1961; Conen, 1962; Schachter, 1962; Scherz, 1962). Precise classification of the extra chromosome can only be done morphologically in excellent preparations. The photographs (Figs. 4.8 and 4.9) show that in these abnormal children three chromosomes of type 21 and two of type 22 can be found.

Identification of chromosome 21 by its satellites attached to a very small short arm has raised and still raises difficulties owing to the possible presence of satellites on one (cf. Fig. 4.9) and exceptionally on the two 22 chromosomes. Some workers discussing this difficulty have suggested calling the disease trisomy (21 or 22).

Such imprecision seems to us dangerous and to take a logical position it is useful to review the facts.

The existence of three satellited acrocentrics reported from the very outset by Lejeune *et al.* (1959g) and Böök *et al.* (1959) has since been widely confirmed.

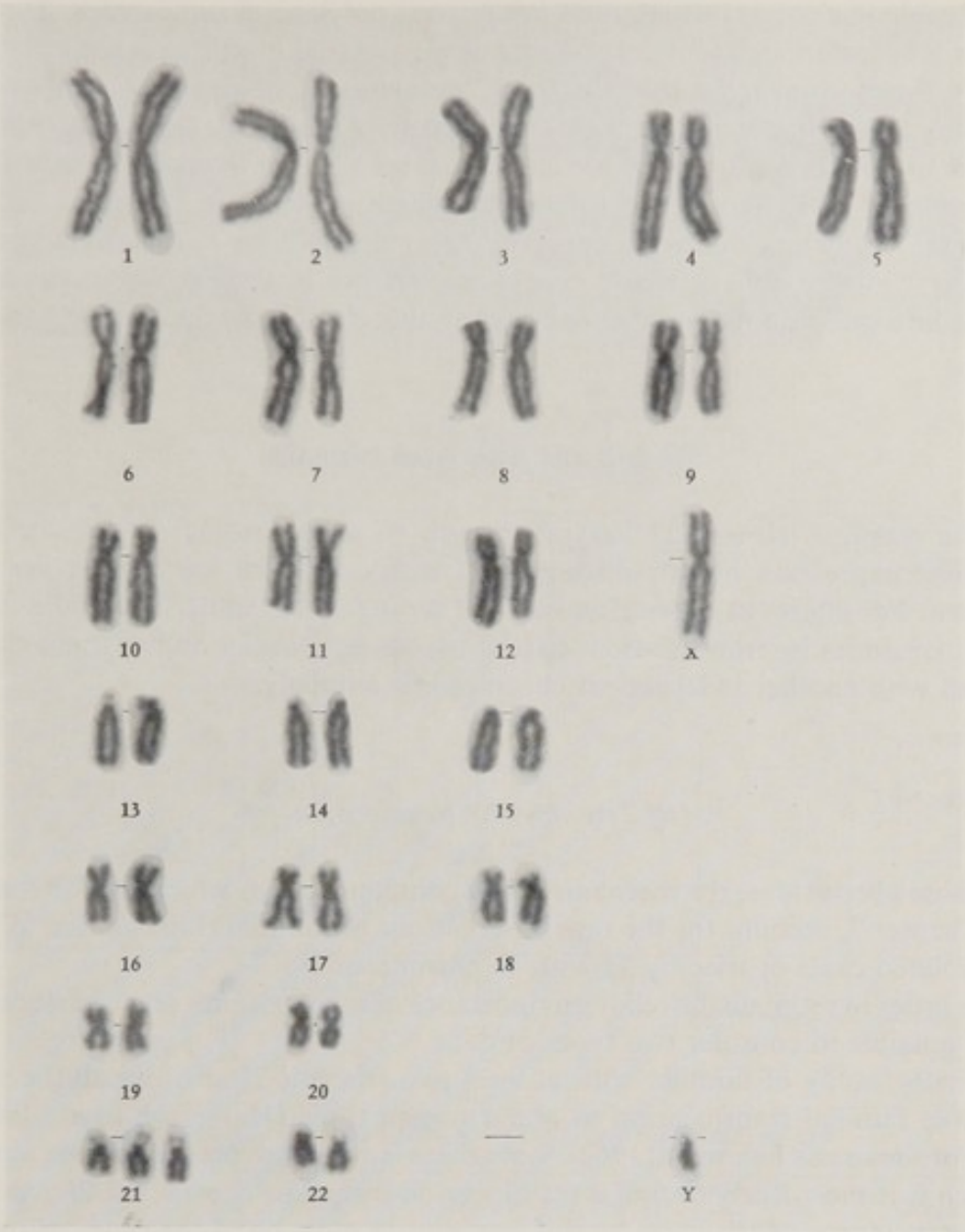


FIG. 4.9. Karyotype of the previous cell. Note the presence of three very similar chromosomes 21. One of the chromosomes 22 has very small satellites and the other none at all.

As we have seen (Chapter 3) the distinction between chromosomes 21 and 22 is always intricate and sometimes impossible. However, as is made clear by Figs. 4.8 and 4.9, the existence of three chromosomes of type 21 appears more probable than that of three of type 22.

This morphologically very probable trisomy is also confirmed by the existence of trivalents at meiosis in subjects with trisomy 21 (O. J. Miller *et al.*, 1960) and also in bearers of translocation 21 ~ D (Hamerton *et al.*, 1961 b) (Fig. 4.10).

Since meiotic association remains the best criterion of genic homology it may be considered that trisomy for chromosome 21 has been demonstrated as far as

is possible in a species which does not possess polytene chromosomes. The disorder is therefore due to an excess of genetic material in other respects normal.

We therefore propose that the term "mongolism" be definitely replaced by trisomy 21, in line with the wish expressed by Allen *et al.* (1961), in order to avoid use of such an unsuitable term. It must also be formally specified that the number 21 defines the chromosome which is found in triplicate in "this disorder". Any new discovery concerning the morphology of this chromosome, even if it allows us to attribute new characteristics to it must not in any event bring into question the number assigned to this element by the Denver commission.

Masked and associated trisomies

The classical trisomy 21 karyotype with 47 chromosomes is not the only possible expression of this disorder and many different karyotypes are now known. For clarity of exposition we shall arrange them under four main headings: trisomies by translocation, partial trisomies, mosaics and trisomies associated with another independent chromosome anomaly.

(a) Trisomies by translocation

These aberrations, the mechanism and consequences of which will be studied in Chapter 7, account for the rare observations of familial transmission as well as isolated cases of trisomy 21 with 46 chromosomes.

In order to estimate the relative importance of these trisomies by translocation it is possible to consider two types of data.

Firstly, study of families with at least two trisomic 21 sibs reveals the existence of familial translocation in about one in three (Hamerton *et al.*, 1961a, b; Forssman and Lehmann, 1962). Since these families are precisely those among which it is most likely to find a translocation it may be considered that the relative incidence of trisomies by this mechanism must be of the order of only a few per cent at most. In fact, sibship recurrence of the anomaly is only about 0·5 in a hundred.

This first indication underestimates the actual incidence of trisomics by translocation since this mode of reasoning *a priori* excludes translocations appearing *de novo* in the affected subject. These *de novo* translocations are, moreover, very frequent (Polani *et al.*, 1960; Cooper and Hirschhorn, 1961a; Gustavson, 1962; Benirschke *et al.*, 1962d; Scherz 1962; Migeon *et al.*, 1962; Brandt *et al.*, 1963b; Hustinx, 1963a; Edwards *et al.*, 1963) and may appear in a family (Walker *et al.*, 1963) or in a sibship (Barnicot *et al.*, 1963) with another trisomic 21 without translocation in this case. They may also form a mosaic: triplo 21 cells with 47 chromosomes and triplo 21 cells with 46 chromosomes by 21 ~ 21 translocation in the same individual (Blank *et al.*, 1963).

The present statistics are very unreliable since in nearly all laboratories the

trisomic 21 patients born to young mothers or belonging to a family already affected by the disease are examined in preference to others.

It is, however, possible to divide the available information into two categories depending on whether the bias is relative and cannot be estimated (Table 4.3) or is systematic (Table 4.4).

Taken as a whole the first statistics suggest that the incidence of translocation is 27 in 652 or 4·1 per cent and the second 13 in 110 or 11·8 per cent. These two values are statistically very different which corroborates the fact that translocations are more common in the progeny of young mothers.

TABLE 4.3

Trisomy 21. General statistics

	Total examined	Trisomy 21	21 ~ 13	21 ~ 21	Others
Benirschke, 1963	73	72	1		
Grouchy, 1963	52	50	–	2	
Hamm, 1963	31	29	1	1	
Hayashi, 1963	79	74	3	–	Two mosaics
Makino, 1963	64	63	1	–	
Mellman, 1963	16	12	2	–	Two mosaics
Palmer, 1963	17	17	–	–	
Institut de Progénèse	111	108	3	–	
Robertson, 1963	35	31	1	3	
Sergovich, 1963	174	165	5	4	
	652	621	17	10	4

TABLE 4.4

Trisomic 21 children born to mothers under 30 years of age

	Total	Trisomy 21	21 ~ 13	21 ~ 21	Others
Lehman, 1962	45	44			
Edwards, 1963	25	21	2	1	
Haylock, 1963	28	22	3	2	
Breg, 1963	12	10	2	3	
	110	97	7	6	

From these figures it may be reasonably considered that the true relative incidence of translocations in trisomy 21 is of the order of a few per cent: from 1 to 2 per cent perhaps, the estimate of 4 per cent representing a definitely too high upper limit because of bias in recruitment of the patients.

These translocations mainly concern acrocentric chromosomes having undergone "centromeric fusion".

As pointed out by Hamerton and Steinberg (1962c) translocations of the type 21 ~ D produce a trisomy in one-third of the children if the mother is the transmitter. On the other hand, in the cases of transmission by the father, trisomies appear exceptional (see Chapter 7).

The same tendency is also found in cases of translocation between small acrocentrics of the type 21 ~ G but the situation here is more difficult to define because of the difficulties of cytological diagnosis of the three possible types: 21 ~ 21, 21 ~ 22 and 22 ~ 22, the effects of which are *a priori* very different.

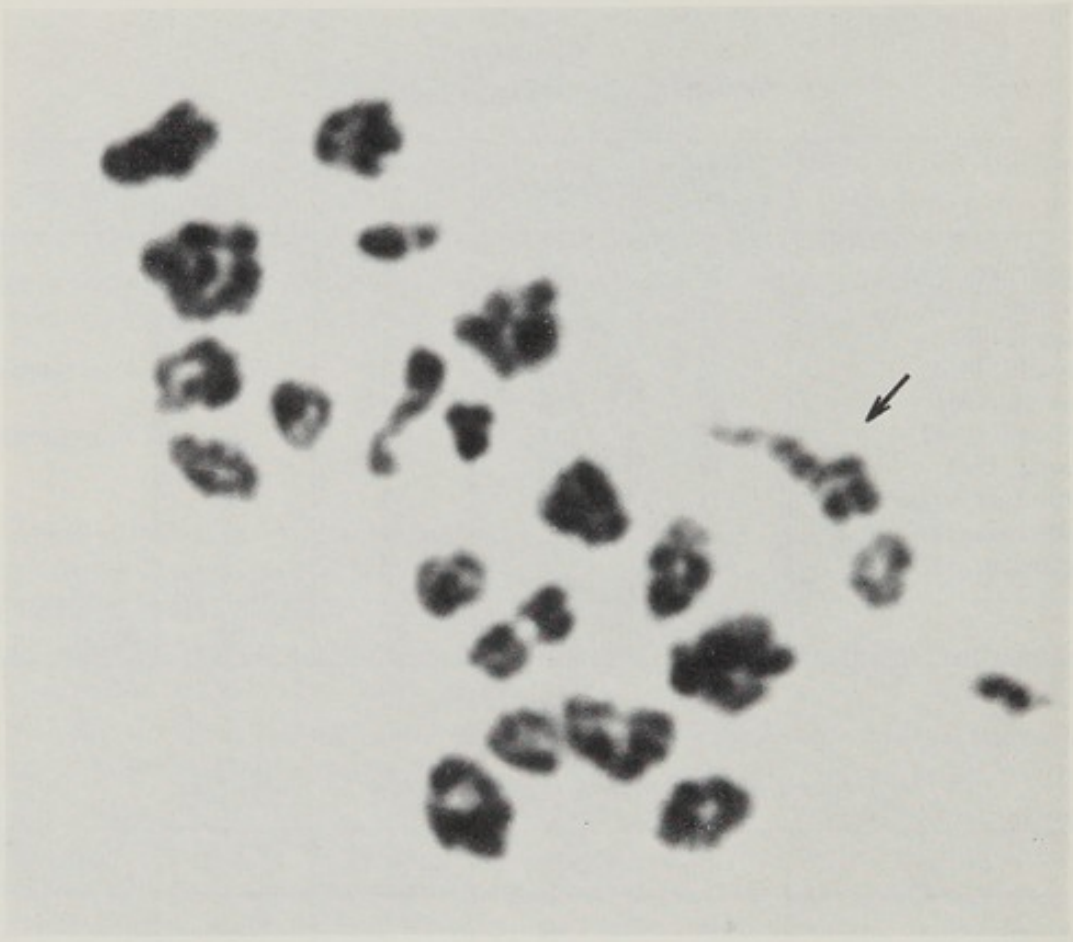


FIG. 4.10. Formation of a trivalent at meiosis in a male subject with 21 ~ D translocation. (Photograph by courtesy of Dr. J. L. Hamerton.)

Apart from these two major types of rearrangement some other karyotypes with 46 chromosomes are known: translocation of a portion of the short arm of chromosome 2 onto a 21 producing a new chromosome 2 ~ 21 resembling a D (J. R. Miller and Dill, 1963) and translocation of two 21 chromosomes producing an acrocentric element of type D instead of the more frequent mediocentric type (Zellweger *et al.*, 1963; Warkany and Soukup, 1963; Institut de Progénèse, No. 1341).

The existence of these trisomies by translocations provides new confirmation of the reality of trisomy 21 and the hypothesis that the disorder results from an

overdosage of genes. In fact, the observations of Hamerton *et al.* (1961 b) showed that during diakinesis in spermatocytes there were 21 bivalents and 1 trivalent in a male subject bearing the translocation 21 ~ D. This association between the two 21 chromosomes (one free and the other translocated) is the best possible cytological proof of the homology between the identifiable 21 and that which is part of the hybrid chromosome 21 ~ D.

(b) Mosaics

The notion of overdosage of genes is also applicable to mosaics in which an individual carries side by side two, or even three, different cell clones overlapping to form a mixed population one with trisomic 21 cells, the other with normal diplo 21 cells.

The relevant published findings are summarized in Table 4.4.

It is very difficult to draw precise conclusions from these figures since it is doubtful whether a percentage (between normal and trisomy 21 cells) calculated from a biopsy is representative of the tissue examined. Moreover, the distribution of the mosaic in different tissues may vary greatly (case of normal blood in the child reported by Clarke [1961]) and the relation between the relative incidence of triplo 21 cells and the malformation of tissue which contains them is actually impossible to establish quantitatively.

We would simply note that borderline individuals, those who show almost normal mental development, are diplo 21/triplo 21 mosaics with a clear predominance of diplo 21.

On the other hand, the two cases of very severe debility show in addition to diplo 21 and triplo 21, tetraplo 21 cells or even perhaps pentaplo 21 which suggests that the relation between the surplus amount of chromosome 21 and the gravity of the genetic imbalance represents an approximation acceptable for further research (Chapter 15).

However, it must be pointed out that Clark *et al.* (1963) reported that the relative incidence of the two populations, diplo 21 and triplo 21, may vary in time. Within 3 years the child described by Clark *et al.*, showed 17 per cent triplo 21 in 1963 as against 32 per cent in 1960.

Mosaicism in one of the parents may cause the recurrence of trisomy 21 within the same family. When the mosaic affects all the tissues of the individual (Smith *et al.*, 1962c; Blank *et al.*, 1962) it is easy to demonstrate, but a mosaic confined to the gonadal tissue is not detectable with present techniques, and the importance of this type of anomaly cannot be directly assessed.

Finally, two cases of complex mosaics with two or even three surplus very small acrocentrics combined with classical trisomy 21 have been reported by Piazzi and Rondinini (1961) and Valencia *et al.* (1963a) in very young children. This type of mosaic strongly suggests a possible initial clonal development.

The existence of monozygotic twins, one normal the other with trisomy 21 (heterokaryotic monozygotism, Chapter 13), may be likened to these mosaics (Lejeune *et al.*, 1962d; de Wolff, 1962). In fact, abnormal segregation of chromo-

TABLE 4.5

Diplo 21/triplo 21 mosaics

Authors	Total number of cells inspected		Cell types as %				Condition of subject
			Diplo 21	Triplo 21	Tetra 21	Others	
Clark *et al.*, 1961 Mother 26 years Father 26 years	Skin Blood	89 44 22	53% 38% 100%	47% 62% 0%			Girl of $2^1/_2$ years. Facial features and general appearance trisomic 21. Typical dermatoglyphs; IQ 100%. Normal intelligence and motor development. Absence of trisomic 21 cells in the blood
Fitzgerald and Lycette, 1961	Blood	100	42%	53%	5%		51-year male. Severe mental deficiency. Diagnosis of mongolism but epicanthus, transverse crease and abnormal ears absent
Gustavson and Eck, 1961 Mother 24 years Father 29 years	2 skin	60	30%	30%		40%	12-year male. Brother died at 6 months, probably trisomic 21. Severe mental deficiency. Well-established diagnosis of epicanthus, furrowed tongue, brachycephalia, squashed nose, hyperlaxity of ligaments and weak muscle tone. Trisomy 21 + a small supernumary of type 19–20 (probably 22 ~ 21)
Nichols *et al.*, 1962a		57	81%	19%			Moderate syndrome but facies positive plus Brushfield spots
Warren *et al.*, 1961	Blood	93	58%	27%	?	15%	Female with typical facies. Annular pancreas. Quotes two cases, one of Lytt's, the other of Conen's
Hayashi *et al.*, 1962 Mother 17 years	Blood	100 18	55% 57%	45% 43%			3-year male. Atypical facies: epicanthus, small and low-set ears, short hands. Speech retardation, but is said to be of normal intelligence
Richards *et al.*, 1962 Young mother	Blood	45	48%	52%			Adult trisomic 21 male with typical severe mental deficiency
Lindsten *et al.*, 1962b	Blood and skin	293	62%	35%		3%	Definite morphological signs but borderline diagnosis. Negative dermatoglyphs. Facies positive, slight mental retardation. Considered normal
Zellweger and Abbo, 1963		59	33%	67%			Child of 3 years. Typical syndrome. Mental deficiency

some 21 during the early blastomeric stages usually leads to a mosaic individual. However, if a separation of the blastomeres into two monozygotic twins occurs before the appearance of the abnormal triplo 21 clone this may result in two individuals genetically identical apart from the presence of trisomy 21 in one of them.

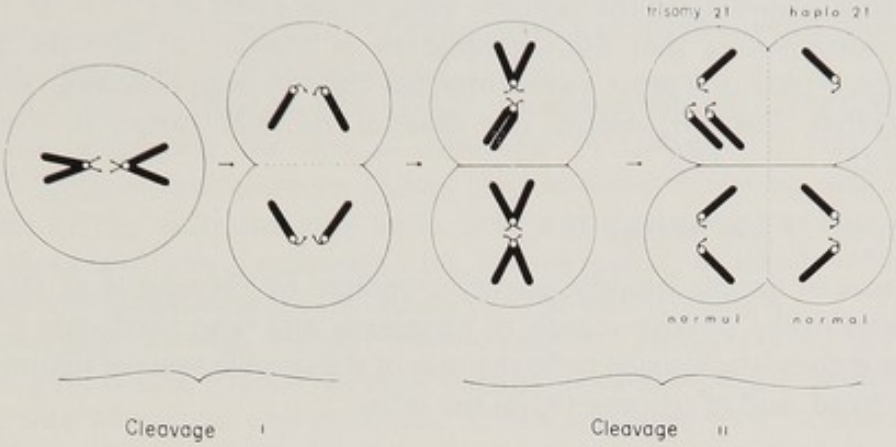


FIG. 4.11. Abnormal segregation of chromosome 21 during the initial cleavages of the zygote. This leads to formation of a mosaic, triplo 21/diplo 21. If there is separation into two distinct embryos the result is heterokaryotic monozygotism: one normal twin and one trisomic 21 twin.

(c) Associations

The chromosome anomaly, trisomy 21, is sometimes associated with other anomalies. Trisomy by translocation (21 ~ 21) or (21 ~ 13) has been mentioned previously and we shall examine the other associations: essentially sex aneuploidies in addition to trisomy 21.

(1) Trisomy 21 and Klinefelter's XXY syndrome

In 1959, Ford *et al.* described the first case of association of chromosome aberrations in a subject presenting a typical Klinefelter's syndrome coupled with the classical trisomy 21 syndrome. The subject, born to a 40-year-old mother had a karyotype of 48 with three 21, two X and one Y chromosomes. This patient again studied by Harnden *et al.* (1960) was to be joined by the patients of Lanman *et al.* (1960), Lehmann and Forssman (1960a) and finally, the twins of Hustinx *et al.* (1961). These monozygotic twins born to a 43-year-old mother were identical boys (probability of monozygoty 99·5 per cent) both carrying 48 chromosomes with the formula 44 A + 21 + XXY.

A comparable case, monozygotic twins both with 48 chromosomes by trisomy 21 with the sex formula XXY, has been observed in our laboratory (Turpin, *et al.*, 1964c). Since, the probability of this triple combination: monozygotism, trisomy 21 and Klinefelter's syndrome, can be evaluated at $1{\cdot}2 \times 10^{-8}$ it seems plausible to imagine the existence of a factor common to these three features as this event has already been observed twice.

(2) Trisomy 21 and triplo-X

A 2·5-year-old child with a retinoblastoma showed in addition trisomy 21 and three X chromosomes (karyotype 48 chromosomes) (Day *et al.*, 1963b). Here too the combination of the three characters, trisomy 21, retinoblastoma and triplo X, was highly improbable, the probability of its appearance by mere coincidence according to the authors, being of the order of $1{\cdot}25 \times 10^{-10}$.

It is worth noting that the combination of trisomy 21 and the haplo X karyotype typical of Turner's syndrome has still not been observed. Van Wijck *et al.* (1964), however, report one case of XO/XX mosaic in a girl with trisomy 21.

(3) Trisomy 21 plus trisomy 18

One case of double trisomy has been reported by Gagnon *et al.* (1962) in a child born in the seventh month of pregnancy and who lived only 8 hr. The complex deformities seemed to be the sum of the specific signs of trisomy 21, on the one hand, and of trisomy 18, on the other.

(4) Trisomy 21 and autosomal translocation

Two cases of free trisomy 21 with 46 chromosomes because of a D ~ D translocation between two large acrocentrics have been reported (Hustinx 1963a; Hamerton *et al.*, 1963a).

(d) Partial trisomies and allied syndromes

Some patients presenting a clinical syndrome similar to that of trisomy 21 but incomplete have been described. Ilbery *et al.* (1961) observed a fairly typical child with normal palmar prints showing two types of cells, some normal with 46 chromosomes, the others with 47 due to presence of a very small chromosome resembling a small acrocentric which had undergone substantial deletion. Likewise, Dent *et al.* (1963a) describe a girl presenting a *forme fruste* of the disease whose cells with 47 chromosomes contained a very small chromosome in excess. This small acrocentric with part of the long arm amputated resembled a Ph^1 chromosome.

Finally pericentric inversion of chromosome 21 was considered by Grey *et al.* (1962) in the mother of a child with trisomy 21 with translocation between two small acrocentrics.

A 4-year-old girl described by Böök *et al.* (1961), presenting malformations of the trisomic 21 type with quite atypical morphology together with moderate mental retardation, had a karyotype with 46 chromosomes with normal sex complement, three chromosomes of type 21 and an apparent monosomy 16. Another explanation proposed by the authors assumed existence of a translocation of the short arms of 16 to 21. The small extra telocentric is thought to be made up of the centromere and satellites of 21 and the short arm of 16, the child then having partial trisomy 21 and partial monosomy 16. This patient is also

cytologically quite close to another with congenital cardiopathy (Böök *et al.*, 1961f) though not very comparable clinically.

Four children clinically atypical but presenting certain characters of the syndrome were reported by Chu *et al.* (1961) and are considered by these authors as probably partially trisomic for chromosome 21 through a very small translocation 21 ~ 22 carried by the mother at the limits of detection. A parallel may be drawn between the difficulty of being certain in these difficult cases and the negative observations of Schmid *et al.* (1961).

The last case reported by Ellis (1962) concerns a mentally retarded epileptic child not displaying the clinical syndrome of trisomy 21. Her karyotype with 47 chromosomes contains a small extra acrocentric having well-developed satellites on the short and long arms. Such a chromosome can only result, as the authors say, from a rearrangement, the complexity of which unfortunately prevents any speculation on the identity of the extra fragment.

To conclude, we shall compare the two cases of Zellweger (1962d), two trisomic 21 girls with a slightly atypical clinical picture, with the patient of Hall (1962) with 46 normal chromosomes. At first described as a "trisomy 21 with normal chromosomes" (Hall, 1963) this patient was diagnosed as suffering from a clinical syndrome distinctly different from that of trisomy 21 (Hall, 1964). These two examples well illustrate the difficulties encountered in clinical diagnosis in certain "borderline cases".

CHAPTER 5

Numerical Autosomal Aberrations
Trisomies 13 and 18

In addition to trisomy 21, two other trisomies are now well established in the sense that many independent examples of them are known and the morphological picture is of sufficient uniqueness to allow a clinical diagnosis of the condition.

These are in order of their discovery trisomy for a chromosome of the group 17–18 described by Edwards *et al.* (1960b) and trisomy for a chromosome of the 13–15 group described by Patau *et al.* (1960b).

For greater simplicity and in accord with the proposals of Gottlieb *et al.* (1962) and Edwards (1963) we shall call these two conditions trisomy 18 and trisomy 13, respectively.

TRISOMY 13

Discovered in 1960 by Patau, Smith, Therman, Inhorn and Wagner, this trisomy for a medium-sized acrocentric of the 13–15 group was rapidly confirmed at first by the same authors, then by many other laboratories. This syndrome has been described in 28 cases in available publications.

As proposed in the preceding chapters and to avoid any ambiguity we shall use the term trisomy 13 to define this condition, 13 denoting the chromosome the presence of which in triplicate produces the disorder.

(a) Habitus in trisomy 13

These are underdeveloped children (average weight at birth 2·3 kg with a standard deviation of 0·4 kg) with multiple malformations and profound mental retardation. Deafness, often mentioned, cannot be authenticated because of the prostration and indifference of the newborn.

The skull is small, mainly through aplasia of the frontal and parietal regions. The eyes are small with true microphthalmia, ranging as far as total anophthalmia in some exceptional cases. When the eyeballs are of normal size an iridocoloboma is nearly always observed with opacity of the cornea or the crystalline lens. Flat angiomas are nearly always noted especially in the frontal and nasal regions.

The ears are malformed. Low implantation of the lobes and the smallness of the mandible are often noted but are much less striking than in trisomy 18.

Finally, very frequently there is a harelip, often bilateral with sometimes almost complete cleft palate (wolf-mouth). In certain cases the nose is completely aplastic, without harelip, giving a cebocephaly.

The trunk is generally not very deformed but hernias are frequent.

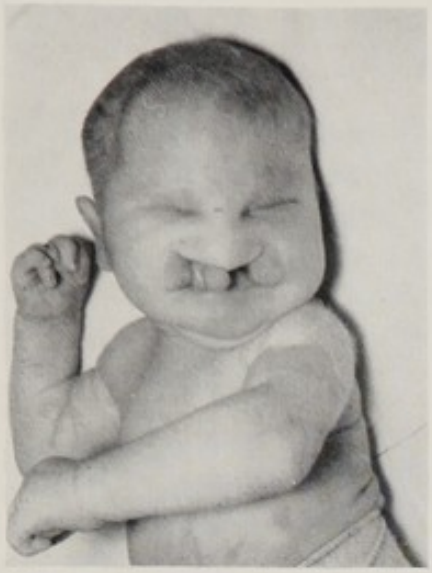


FIG. 5.1. Boy with trisomy 13 (Institut de Progénèse, No. 789).

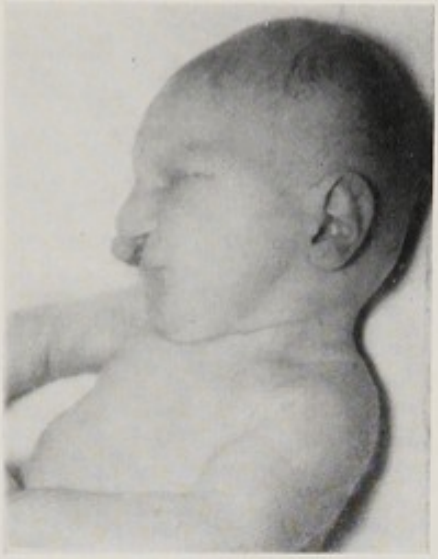


FIG. 5.2. Same boy with trisomy 13 (Institut de Progénèse, No. 789).

In the extremities one observes flexion of the hands and fingers which often cannot be straightened and sometimes clinodactyly of the fifth digit recalling the "hand" in trisomy 18. Polydactyly of the hands and feet is an almost regular feature, the extra fingers or toes may be completely developed (hexadactyly) or be reduced to a simple side stump. Fused or webbed fingers and toes are frequent. Deformity of the feet, *pied en piolet*, is very characteristic. Finally, the nails are narrow and hyperconvex in the shape of an inverted "gutter".

(b) Internal malformations

Heart defects are extremely common (77 per cent): interventricular communication (53 per cent), patent ductus arteriosus (35 per cent) and interauricular communication (14 per cent). Dextrorotation of the heart and stenoses and malpositioning of the large vessels have also been reported.

The kidneys may be malformed and the intestine developed abnormally (ectopic colon, segmental duplication of the small intestine).

In girls, the uterus is often bicornuate or even entirely partitioned and in boys, cryptorchidism and abnormal extension of the scrotum on the lower aspect of the penis are common. Partial agenesia of the epididymis has been noted.

The cerebral malformations appear to be much more extensive than in trisomy 18. The most constant is aplasia of the olfactory bulbs and the trigone (sometimes also the optic tract) giving typical arhinencephalia.

TABLE 5.1

Main stigmata of trisomy 13

		Frequency	Number of cases analysed
	External deformities		
Head and neck	Deformed ear lobe	0·96	25
	Eye anomalies	0·93	26
	Microphthalmia or anophthalmia	0·70	27
	Iridocoloboma	0·68	19
	Opacities of the cornea or crystalline lens	0·75	20
	Hypotrophy of the frontal or temporal eminences	0·82	22
	Cleft palate or harelip	0·78	27
	Harelip alone	0·07	27
	Cleft palate alone	0·11	27
	Cleft palate + harelip	0·60	27
	Angioma of the face or scalp	0·67	24
	Receding jaw	0·39	18
Trunk	Hernias	0·47	17
	Cryptorchism with or without anomaly of the scrotum	0·64	11
Limbs	Medio-palmar triradius t''	1·00	10
	Transverse palmar crease	0·80	20
	Extra fingers or toes	0·77	26
	Flexion of fingers and hand	0·62	21
	"Rocker bottom" foot	0·60	15
	Hyperconvexity of the nails in the form of a "gutter"	0·60	15
	Possible retroflexion of thumb	0·36	11
	Internal deformities		
	Cardiopathy	0·77	26
	Interventricular communication	0·53	19
	Patent ductus arteriosus	0·35	17
	Interauricular communication	0·14	14
	Renal deformities	0·47	19
	Absence or atrophy of the olfactory lobes (arhinencephalia)	0·47	17
	Bicornuate or double uterus in girls	0·30	15
	General involvement		
	Severe mental deficiency	1·00	18
	Deafness (or no response to sound stimulus)	0·80	15
	Convulsions or attacks of trembling	0·44	16
	Hypotonia	0·44	10

Relative frequencies estimated solely from cases with explicit presence or absence of the trait considered and from the findings of:

Atkins and Rosenthal, 1961; Bühler *et al.*, 1962; Conen *et al.*, 1962a; Ellis and Marwood, 1961; Ferguson-Smith, 1961; Gustavson *et al.*, 1962; Koenig *et al.*, 1962; Lubs *et al.*, 1961; M. Miller *et al.*, 1963 and J. Q. Miller *et al.*, 1963b; Northcutt, 1962; Lafourcade *et al.*, 1964; Rosenfield *et al.*, 1962; Schade *et al.*, 1962; Sergovich *et al.*, 1963; Shärer *et al.*, 1962; Shaw and Nishimura, 1961; Smith *et al.*, 1963; Townes *et al.*, 1962 a; Warbourg and Mikkelsen, 1963.

This lesion appears so important that some workers consider that the majority of the commonly described arhinencephalies probably derive from trisomy 13. Warbourg and Mikkelsen (1963) even proposed a new eponym for this disorder: the Bartholin–Patau syndrome.

It seems to us more practical and reliable to retain the chromosome-based terminology.

(c) Dermatoglyphic syndrome

Uchida *et al.* (1961c) were the first to report the existence of two dermatoglyphic signs almost constant in this condition: the transverse palmar crease and the mediopalmar position (position t″) of the axial triradius.

Of 20 cases covered in the present statistics (cf. Table 5.1) 16 showed a unilateral or more often bilateral transverse crease. In 10 cases, for which the palmar prints were fully analysed, the axial triradius was always in position t″.

The dermatoglyphic patterns of the hypothenar eminence are still not very clear.

It should be noted that these two stigmata: transverse palmar crease and triradius t″ are very frequent in trisomy 21 and this pattern in the two disorders illustrates the difficulties of using malformation symptoms in evaluating the gene contents of the chromosomes. Finally, Uchida *et al.* (1962) describe on the sole of the foot presence of an arch involving all the toes. This formation is thought to be very typical of trisomy 13.

(d) Conditions for appearance of this trisomy

The effect of maternal age reported as early as 1961 by Smith *et al.* is difficult to demonstrate in this trisomy.

As Table 5.2 shows, the majority of children with trisomy 13 were born to young mothers (under 35 years) but it is difficult to specify at the present moment the statistical significance of the excess of mothers over 40 years of age observed.

It seems, as in the cases of trisomy 18, that a bimodal distribution mirrors the facts. This would imply the notion of two causal categories: one part of the trisomy 13 cases being independent of maternal age, the other part being very sensitive to it.

It should be noted that the fact that girls are more frequently affected, as reported by Ferguson–Smith (1962) is not borne out on a larger sample (15 ♀ against 11 ♂) nor does the distribution of sexes as a function of the mother's age show the anomaly so blatant in trisomy 18.

(e) Chromosomal diagnosis

As was recognized from the outset by Patau *et al.* (1960b), the additional chromosome is a medium-sized acrocentric of group 13–15 (cf. Figs. 5.3 and 5.4).

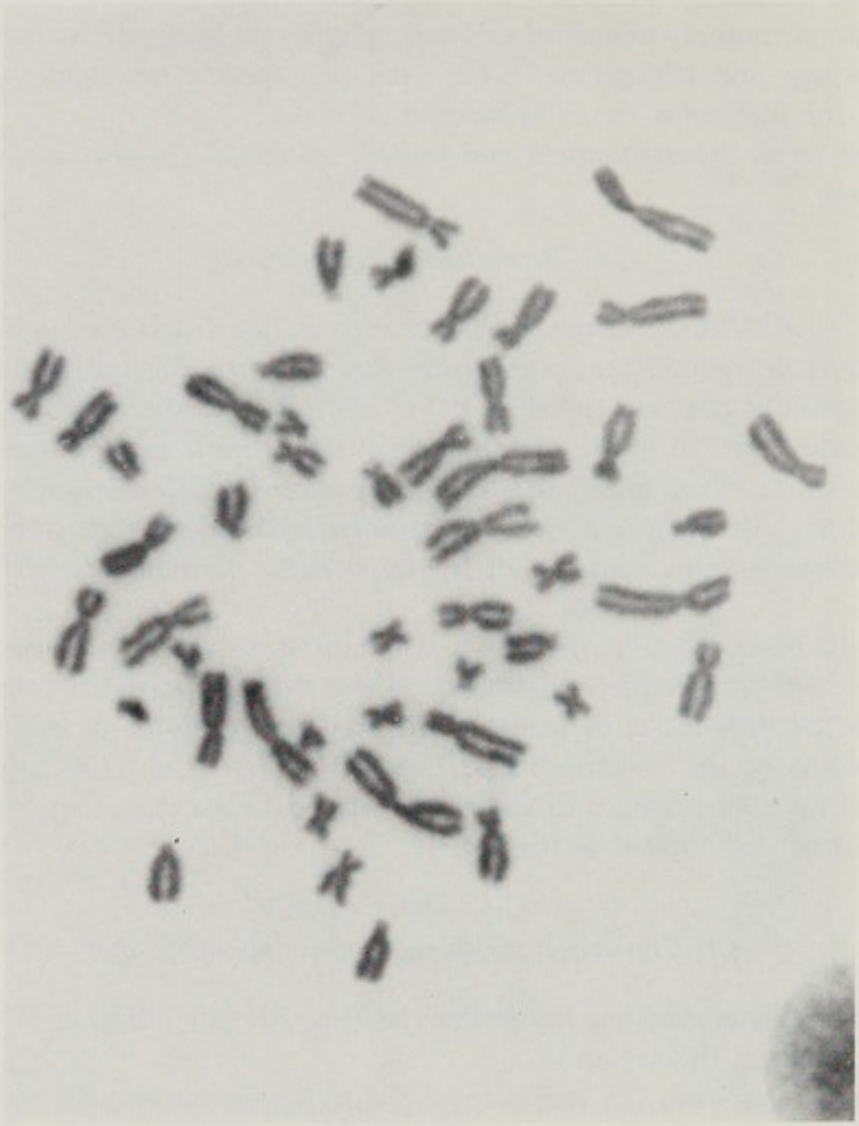


FIG. 5.3. Cell of a boy with trisomy 13 (Institut de Progénèse, No. 789).

We shall not dwell on the difficulties of the individual identication of these chromosomes nor on the weakness of the criteria of identification proposed in the Denver system (cf. Chapter 3). From our personal experience it seems that the supernumary is the carrier of small well-developed euchromatic arms and has no satellites. To be more exact, we find among these subjects three acrocentric chromosomes instead of two, corresponding to these characteristics. As stated above, the number 13 may be deliberately assigned to this chromosome and even if advances in cytology permit unequivocal identification this number must be retained definitively.

It must be made quite clear that the designation trisomy 13 in no way implies that we are now able to pinpoint this chromosome.

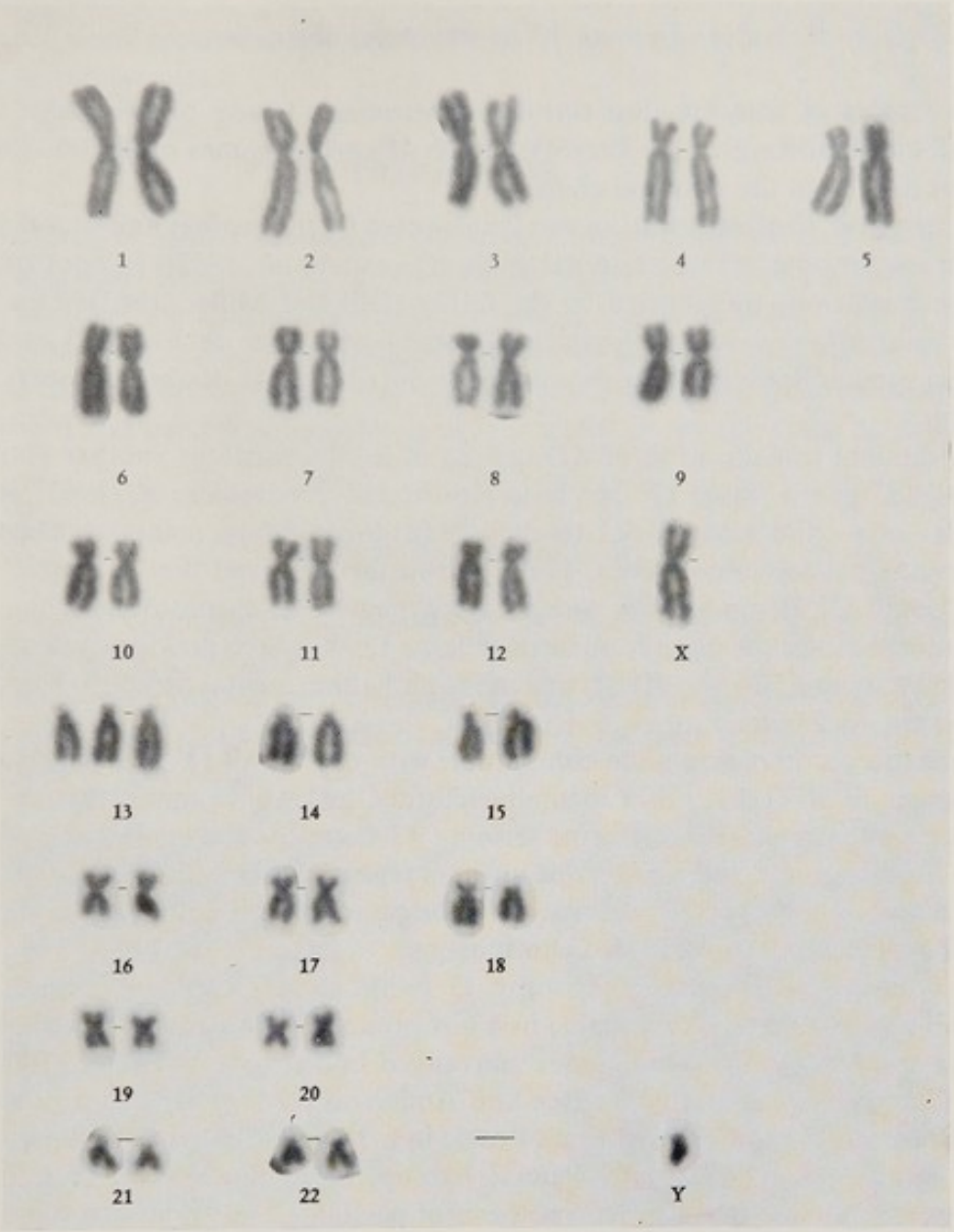


FIG. 5.4. Karyotype of a boy with trisomy 13 (Institut de Progénèse, No. 789).

TABLE 5.2

Distribution of 25 cases of trisomy 13 by age of mother and sex of child

	15–19	20–24	25–29	30–34	35–39	40–44	45 +	Total
M	0	1	5	0	1	3	1	11
F	0	1	3	6	1	3	0	14
		2	8	6	2	6	1	25

In three other cases (3 girls) the mother's age was not indicated

(f) Trisomy 13 by structural aberration

Two cases of translocation through centromeric fusion of two large acrocentrics are known giving a karyotype with 45 chromosomes in the transmitter parent and 46 in the trisomic child.

In one case, the translocation was transmitted by the mother and found again in her own mother and a maternal uncle (Oikawa *et al.*, 1962). In another, the translocation was transmitted by the father (Dill and Miller, 1963). This type of translocation is morphologically identical with that in Fig. 7.15 and the rearrangement does not seem in practice to entail the loss of chromosomal substance.

A terminal translocation of a fragment of a telocentric to another giving a "small 13" and a "large 13" has been reported by Jacobsen *et al.* (1963), in the mother of a child with partial trisomy 13 (iridocoloboma, mental retardation, transverse palmar crease, etc.). This child in fact received the "large 13" and two "small 13" chromosomes with a karyotype with 47 chromosomes.

A morphologically quite comparable "large 13" has been previously described by Delhanty and Shapiro (1962) in a microphthalmic mental defective who died at the age of 3 years.

An extra chromosome quite comparable with the "small 13" was observed by Zellweger *et al.* (1962c) in a mentally retarded girl whose malformation syndrome was strongly suggestive of trisomy 13 (harelip, cleft palate, abnormal ears, haemangioma, etc.). The same authors report another clinically suggestive case whose sole karyotypic anomaly was the presence of a quite well-developed small short arm on one (13–15) chromosome.

A syndrome suggestive of trisomy 13 (with, in addition, absence of left thumb and rudiment on the right) in a girl presenting five normal acrocentrics plus a small acrocentric sometimes rearranged in a ring was reported by Bain and Gauld (1963). Finally, Wallace and Anderson (1963) observed a probable translocation from a (13–15) to a (4 or 5) in a 34-year-old anophthalmic mentally retarded female with a bilateral harelip, fused fingers and toes and a transverse palmar crease. The hypothesis of partial trisomy was also suggested here.

A case of 46/47 mosaic through presence of a large extra acrocentric has been reported by Warkany *et al.* (1962c) in a malformed subject presenting an incomplete trisomy 13 syndrome.

(g) Combined forms

Two cases of association of trisomy 13 with a trisomy for a small unidentified acrocentric 21 or 22, or a fragment of another chromosome, have been reported, one in a boy (Gustavson *et al.*, 1962), the other in a girl (Becker *et al.*, 1963). Simple summation of the symptoms of each of the two chromosomal syndromes (for example, trisomy 13 and trisomy 21) is not obvious in these two cases.

Finally, Patau *et al.* (1961e) observed a Turner's syndrome (haplo X) in a girl whose sister presented a typical trisomy 13; no chromosome anomaly is reported in the parents.

TRISOMY 18

Discovered in 1960 by Edwards, Harnden, Cameron, Crosse and Wolff, trisomy 18 appears to be more frequent than trisomy 13. The following tables were drawn up from the analysis of about 50 already published cases but the total number observed in the various laboratories is certainly above the 100 mark.

Right from birth the morphological anomaly of the child is conspicuous by generalized and particular symptoms as in trisomy 21 though differing greatly.

(a) Habitus in trisomy 18

The clinical diagnosis of the condition is relatively easy for the experienced clinician (Figs. 5.5, 5.6 and 5.7).

These are small infants born after a pregnancy often complicated by hydramnios (7 of 17 cases) and a small placenta. The weight at birth is low (2·41 $\pm$ 0·51 kg on average in 35 cases).

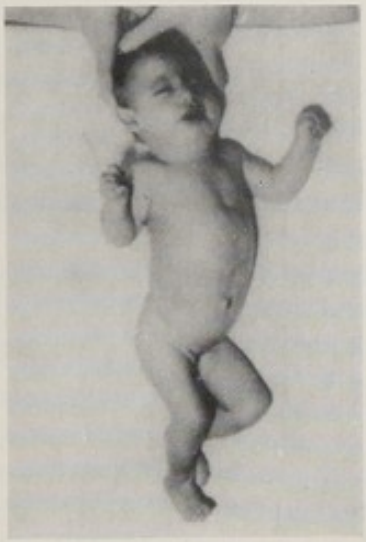


FIG. 5.5. Frontal view of a girl with trisomy 18 (Institut de Progénèse, No. 746).

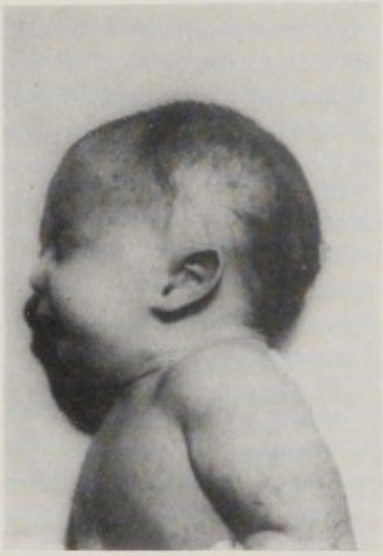


FIG. 5.6. Same girl with trisomy 18 viewed from side (Institut de Progénèse, No. 746).

The skull shows an anterio–posterior elongation (prominent occiput) and a relative lateral flattening. The bridge of the nose is sometimes broad and flat but more often the nose is sharp or even prominent, the ears are very low set and often the pinna is poorly shaped or frankly malformed. Finally, the small lower jaw gives a receding chin suggestive of Franceschetti's disease. In the chest, relative aplasia of the sternum can be seen. The pelvis is narrow, often associated

with congenital dislocation of the hips. Other features, webbed neck, harelip, palpebral ptosis, etc., are occasionally noticed. One also notices a hypotonia at birth which may secondarily give way to moderate hypertonia. On the upper limbs, very typical lesions exist in nearly all cases. The fingers are clenched, which

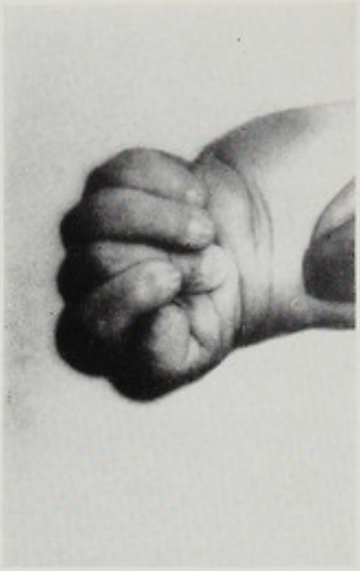


FIG. 5.7. Hand demonstrating the special arrangement of flexed fingers.

may cause rapid maceration of the flexion creases of the palm. Marked deviation of the index and little fingers towards the median line is noted, these two fingers overlapping respectively the middle and the ring finger. This arrangement appears to be particularly typical. The infant holds his arms raised on each side of the head in a supplicant position.

In the lower limbs it is often possible to note an anomaly of the hips (limited abduction or even congenital subluxation) and the existence of club foot of quite varied type is noted in half the cases. Likewise, the first toe is very often short and retracted and syndactly of the second and third toes is very frequent, sometimes also seen in the fingers.

Finally, two major signs complete the picture, the infants are puny, do not gain weight and present signs of very profound mental debility. Their viability is extremely low with the average age at death $2{\cdot}9 \pm 1{\cdot}2$ months for 11 boys and $4{\cdot}2 \pm 0{\cdot}8$ months for 31 girls. The oldest child was 23 months at the time of examination.

(b) Internal malformations

The majority of the deaths are due to aspiration pneumonia or acute heart failure. Congenital cardiopathies are in fact the rule in this disorder since out of 45 cases in Table 5.3, 39 presented grave heart defects.

Interventricular communication is by far the most common malformation

TABLE 5.3

Main stigmata of trisomy 18

	Frequency	Number of cases analysed
External deformities (trisomic 18 habitus)		
Malformed low-set ears	1·00	48
Micro-retro-gnathism	1·00	45
Skull elongated in the anterio-posterior direction with prominent occiput	0·89	38
Narrow palpebral fissures	0·47	36
Webbed neck or exaggerated looseness of the skin of the neck	0·38	37
Harelip with or without cleft palate	0·19	41
Short sternum, shield thorax, laterally shifted nipples	0·83	35
Narrow pelvis, with or without malformation of the hips (luxation)	0·65	31
Hernia	0·53	36
Flexion of the fingers with 2nd and 5th overriding the 3rd and 4th	0·96	47
Hyperextension or shortness of the 1st toe	0·73	34
Club foot, varus, talus, etc.	0·50	38
Syndactyly or palmature of the toes or fingers	0·24	41
Internal deformities		
Congenital cardiopathy	0·91	45
Interventricular communication	0·85	34
Interauricular communication	0·36	28
Patent ductus arteriosus	0·56	32
Renal deformities	0·58	31
Meckel's diverticulum	0·41	27
General disturbances		
Severe mental deficiency	1·00	36
Marked hypotrophy	0·93	41
Hypertonia	0·70	34

Relative frequencies estimated solely from cases with explicit presence or absence of the trait considered and from the findings of:

Brown *et al.*, 1963; Crawfurd, 1961b; Edwards *et al.*, 1960b; Edwards, 1963b; Gagnon *et al.*, 1961; German *et al.*, 1962e; Gottlieb *et al.*, 1962; Hecht *et al.*, 1963b; Hansen *et al.*, 1963; Holman *et al.*, 1963; Koenig *et al.*, 1962; Koulischer *et al.*, 1963; Lejeune *et al.*, 1963f; Nakagome *et al.*, 1963; Institut de Progénèse, 1963; Rosenfield *et al.*, 1962; Smith *et al.*, 1962b; Steinberg and Jackson, 1963; Trowell and Hilton, 1963; Townes *et al.*, 1963; Uchida *et al.*, 1962a; Voorhess *et al.*, 1962; Weiss *et al.*, 1962; Van Wijck *et al.*, 1961a.

(29 of 34), patent ductus arteriosus coming second (18 of 32) followed by interauricular communication (10 of 28) and coarctation of the aorta (4 of 23).

A "bicuspid" anomaly of the pulmonary valve has been reported several times (Gottlieb, 1963).

This extreme frequency of congenital heart conditions very probably accounts for the poor viability of the children affected and suggests that amongst neonatal deaths, or even in the stillborn, the frequency of trisomy 18 is perhaps higher than we at present suspect.

The personal case, whose photograph and karyotype are presented and who died at 1 month, makes clear the need for early examination of the newborn in order to detect this type of trisomy.

Other malformations

Umbilical and inguinal hernias are very frequent as are diaphragmatic hernias. Malposition of the colon is frequent as are renal malformations (polycystic kidney, horseshoe kidney, etc.), presence of a Meckel's diverticulum and sex anomalies in the girl (hypertrophy of the clitoris, abnormal ovaries).

(c) Dermatoglyphic syndrome

Reported in 1961c by Uchida *et al.*, and confirmed by the same authors in 1962c, by Penrose in 1963 and by Uchida and Soltan, 1963, the dermatoglyphic syndrome in trisomy 18 appears to be very characteristic.

On the fingers we note an extraordinary frequency of arches, that is, instead of forming a whorl or a loop, the ridges regularly arrange themselves on each other like geological strata topping an antecline. Arches are infrequent in normal subjects and over 80 per cent show no arch, whereas of the 18 subjects with trisomy 18 studied by Uchida *et al.*, (1962), 17 had at least six and some showed only arches on the fingers. Although this feature may be encountered among normal subjects and although it has been reported in XXXXY individuals, the presence of over six arches is extremely suggestive (less than 2 per cent in the normal population).

Moreover, clinodactyly of the fifth digit and the presence of a single flexion crease at the level of this finger also seems to be very frequent (8 of 13 individuals examined). This symptom appears to be identical with that of the brachymesophalangy in trisomy 21. Finally, a transverse palmar crease usually unilateral, is sometimes encountered without it at present being possible to estimate its frequency.

(d) Conditions for appearance of this trisomy

Frequency of the disease

Differently assessed by different authors, the incidence of trisomy 18 is not yet known with precision.

However, according to the findings of Hecht *et al.* (1964) (two cases in 999 births at the University Hospital of Seattle, Washington) and those of Heinrichs *et al.* (1963) (two cases of 617 births at Watertown Hospital, South Dakota) it appears that the disorder may be just as frequent as trisomy 21.

A provisional estimate of one to two per thousand may be considered reasonable.

Age of mother

The appearance of the disease seems to be distinctly influenced by the age of the mother. In 50 recorded cases the mean age of the mothers at birth was 34·7 years with a standard deviation 8·4 and a mean error of 1·1.

However, it should be noted that there may exist a not inconsiderable number of cases of trisomy 18 independent of maternal age as noted by Hecht *et al.* (1964).

There seems, in fact, to be a frequency peak at about 20 to 29 years which corresponds to the normal period of maximum fertility (cf. Table 5.4). The hypothesis of a bimodal distribution cannot be formally advanced from the limited number of cases available but it does however seem to be corroborated by the abnormal distribution of the sexes in this disorder.

TABLE 5.4

Distribution of 53 cases of trisomy 18 by age of mother and sex of child

	15–19	20–24	25–29	30–34	35–39	40–44	45 +	Total
M	0	3	3	2	2	1	3	14
F	0	6	4	4	8	13	4	39
		9	7	6	10	14	7	53

Based on cases reported by:

Brown *et al.*, 1963; Conen and Erkman, 1963; Crawfurd, 1961b; Edwards *et al.*, 1960b; Edwards, 1963b; Gagnon *et al.*, 1961; German *et al.*, 1962e; Gottlieb *et al.*, 1962; Gropp and Hole, 1963; Hecht *et al.*, 1963b; Hansen *et al.*, 1963; Haylock *et al.*, 1963; Holman *et al.*, 1963; Koenig *et al.*, 1962; Lejeune *et al.*, 1963f; Nakagome *et al.*, 1963; Pfeiffer and Huther, 1963; Institut de Progénèse, 1963; Rosenfield *et al.*, 1962; Smith *et al.*, 1962b; Steinberg and Jackson, 1963; Trowell and Hilton, 1963; Townes *et al.*, 1962 and 1963; Uchida *et al.*, 1962a; Voorhess *et al.*, 1962; Van Wijck *et al.*, 1961a.

Sex ratio of patients

Ferguson–Smith (1962b) noted in 31 cases clear predominance of girls (22 ♀ as against 9 ♂). The information provided in Table 5.4 confirms this predominance: 39 ♀ as against 14 ♂ ($\chi^2 = 11{\cdot}6$ for one degree of freedom).

The distribution of the patients as a function of the age of the mother shows a very curious irregularity. Before the mother has reached 35 years the sex ratio is already abnormal (14 ♀ as against 8 ♂) but after 35 years predominance of girls becomes enormous (25 ♀ as against 6 ♂).

A possible explanation of this dual phenomenon may be found in the notion of supplementation by the mother of the abnormal metabolism of the trisomic child. Maternal age would lower this faculty and the male sex more sensitive than the female would be preferentially eliminated.

In other words, two contradictory factors would be at work because of age:

(a) increase in the frequency of chromosome anomalies;
(b) reduction in the probability of survival of the foetus; the male sex being primarily affected.

A very serious disorder such as trisomy 13 causing a severe selection might thus be below the probable survival threshold for both sexes. This would account for a normal sex ratio and a barely apparent maternal age effect.

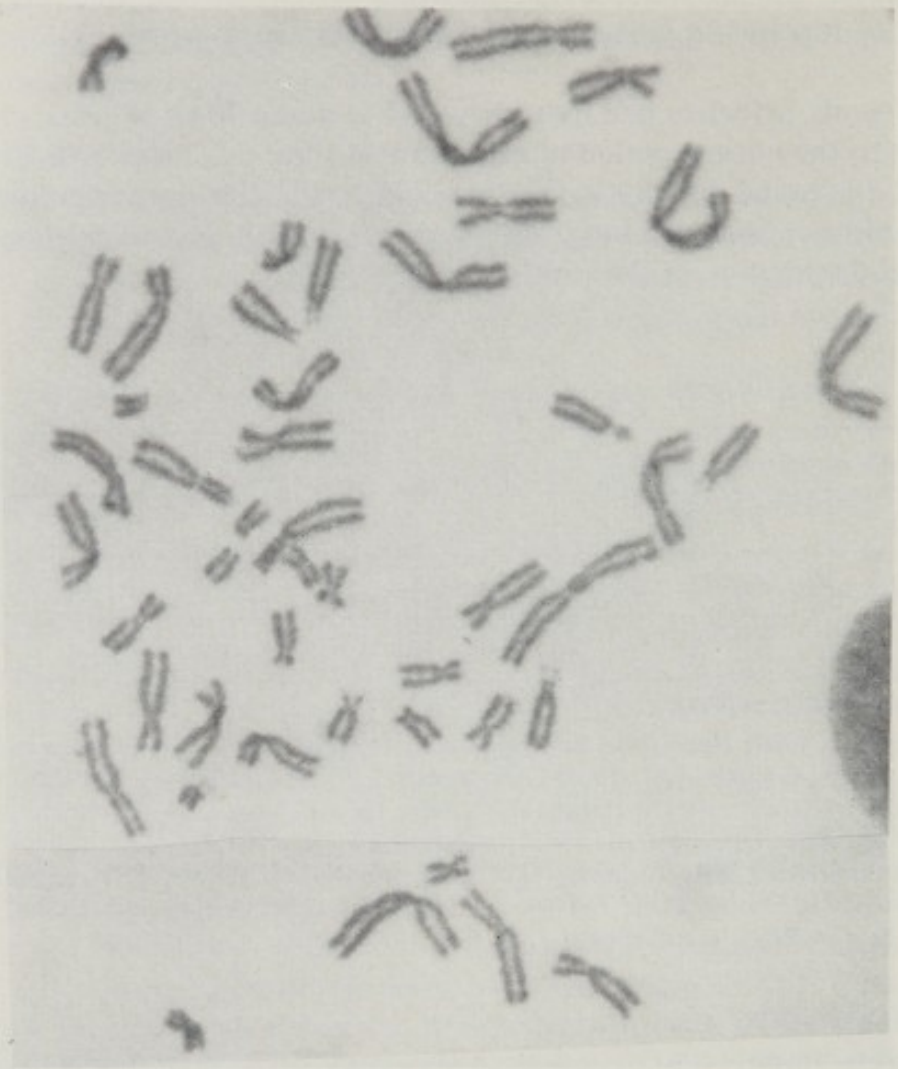


FIG. 5.8. Cell of a female with trisomy 18 (Institut de Progénèse, No. 1089).

An appreciably less serious disorder such as trisomy 18 might reveal a phenomenon of differential selection, whereas a disorder permitting much more satisfactory survival such as trisomy 21 would be virtually unaffected by differential selection.

It is also possible that in the case of trisomy 18 this differential selection has a physiological component arising from the impact of trisomy 18 on sexual development itself. Several cases of abnormal or aplastic ovaries have in fact been reported in trisomic 18 girls.

(e) Chromosomal diagnosis

Described by Edwards *et al.* (1960b) as a trisomy for chromosome 17 this condition which no doubt represents a trisomy for the same chromosome in all cases is considered as a trisomy 18 by Smith *et al.* (1962b), Patau *et al.* (1961f, 1962) and by Uchida *et al.* (1962a). On the other hand German *et al.* (1962e),

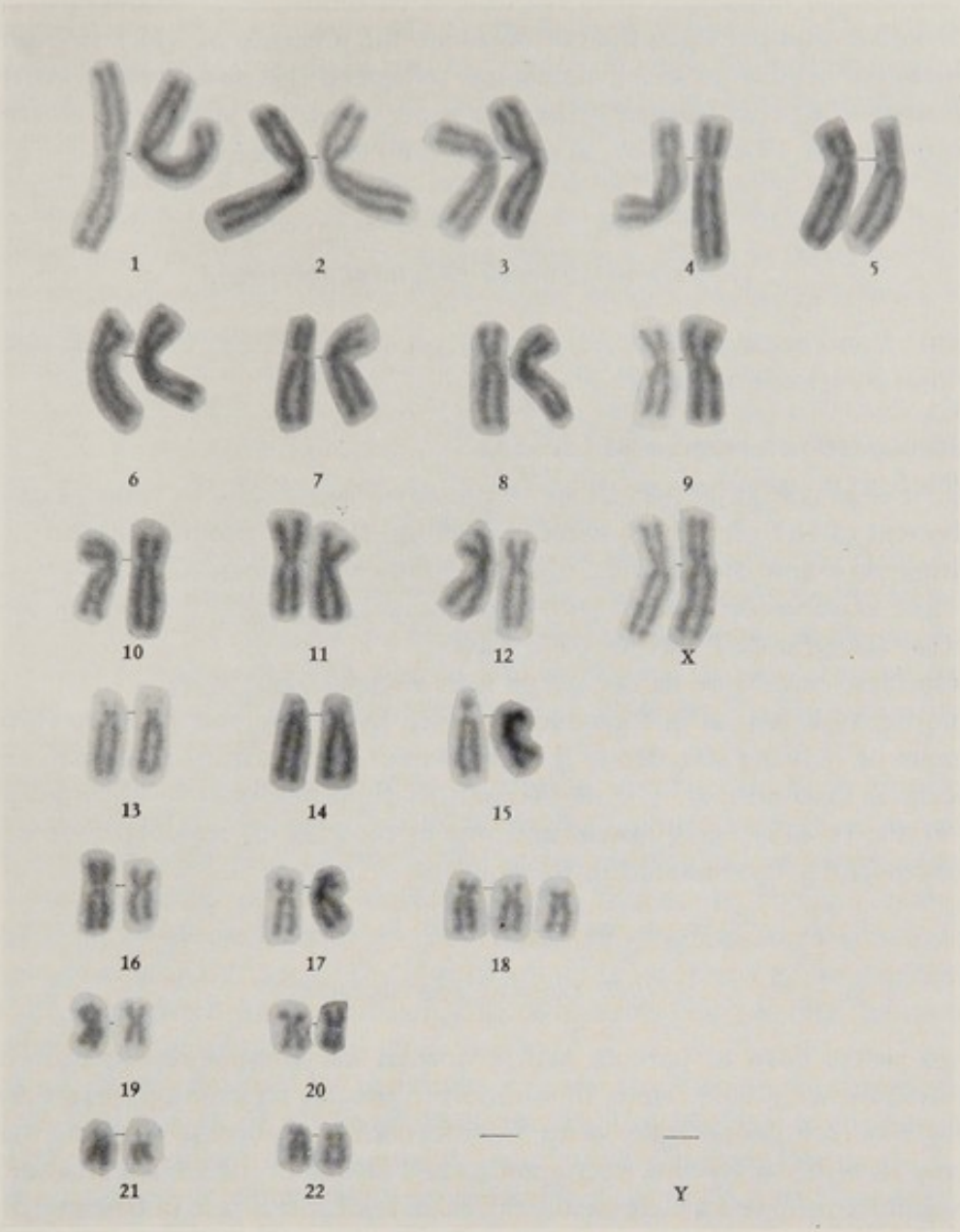


FIG. 5.9. Karotype of a female with trisomy 18. No. 1089.

Crawfurd (1961) and van Wijck (1961 a) hover between 17 and 18. Recently, Edwards (1963), Aherne *et al.* (1962) and Gottlieb (1962) independently related the syndrome to a trisomy 17.

In the personal cases we have studied the most plausible diagnosis appears to be that of trisomy 18 (Figs. 5.8 and 5.9). This discussion reflects the difficulties of diagnosis encountered in the individualization of chromosomes 17 and 18 and the hypothesis of unequal crossing over between 17 and 18 (Muldal, 1961) does not appear necessary for the explanation of these difficulties. It appears advisable for the time being to name the disorder trisomy 18 pending final cytological precision, 18 signifying a chromosome of type 17 or 18.

A reasonable suggestion as in the case of chromosome 21 would be to apply the definitive number 18 to the chromosome the presence of which in triplicate produces the syndrome and if necessary, to amend the classification based on the Denver code if it turns out that the supernumary is larger and more submedian than the "Denver 18", and would then be a "Denver 17".

(f) Trisomy 18 with structural aberration

Apart from the cases of typical trisomy reported in Table 5.3, some cases of trisomies by translocation are known.

Translocation to an acrocentric (13–15)

Hecht *et al.* (1963a) report a case of trisomy 18 syndrome by translocation of a fragment of 18 to the short arm of a medium-sized acrocentric (group 13–15) resulting in a new metacentric, medium-sized chromosome. The karyotype of this child is quite comparable with that of another patient observed in our laboratory (Institut de Progénèse, No. 444).

Both these cases were from parents with a normal karyotype.

Another type is seen in the child observed by Brodie and Dallaire (1963) a fragment of 18 being attached to the long arm of a 13 resulting in a much longer acrocentric than normal. The mother of this child had only 45 chromosomes, one of the (17–18) being absent and one of the (13–15) replaced by the very long acrocentric encountered in the child.

Other types of rearrangement

Two sisters born to parents with a normal karytotype were described by Gustavson *et al.* (1962). Here, in addition to mental retardation, severe hypotrophy was recorded, coupled with a malformation syndrome recalling that of trisomy 18 with low set ears, micrognathia and flexion of the fingers. In addition, these children showed a cleft palate and relative deafness rare in trisomy 18 but more frequent in trisomy 13, the most striking symptoms of which were, however, absent—microphthalmia, haemangiomata, convulsions, harelip and polydactyly. The additional chromosome (identical in the two children) is a little large for a small acrocentric and may well represent a partially amputated chromosome 18 which would accord with the clinical observation of a relatively atypical trisomy 18 syndrome.

A brother and his sister described by Vislie (1962) differed clinically and cytologically from the preceding cases.

The two mentally retarded children both have slit eyes due to an epicanthus, a well-developed forehead, low-set ears and very marked hypotonia. Moreover, the girl shows a transverse palmar crease on the right. Morphologically very similar, they differ greatly from the trisomic 21 habitus.

Their identical karyotypes show a small extra acrocentric not identified by the

authors. In the mother, the absence of a large acrocentric and of an 18 chromosome coupled with the presence of an extra acrocentric (comparable with that of the children) and a median-sized chromosome with very distal centromere suggests the possibility of a 13 ~ 18 balanced translocation in the mother leading to partial trisomy 18 or 13 in the children.

A chromosome 4 with its long arm increased by one-third of its length has been observed by Gagnon *et al.* (1963) in a female infant whose malformations strongly recall the trisomy 18 syndrome. However, it should be noted that this child presented unusual malformations of the hands: absence of thumbs and of arches in the fingerprints.

A very small extra metacentric is reported in two patients, a boy of 2 years (Frøland *et al.*, 1963) and a stillborn girl (Lele, 1963a). These two cases evoking the malformation syndrome may correspond to a trisomy for a chromosome 18 having lost part of its long arm and producing a much more marked deletion than in the cases reported by Crawfurd (1961).

A large subtelocentric was observed by Rohde *et al.* (1963c) in a child presenting a very evocative clinical syndrome. The presence of only one normal 18, the other being replaced by the subtelocentric element, led the authors to consider a translocation of a part of a chromosome 18 onto the long arm of another chromosome 18. The parents of this child were said to have normal karyotypes.

Excess chromosome material on a small acrocentric has been reported by van Wijck (1961a). The patient aged 20 years presented only a few anomalies: low-set malformed ears, webbed neck, pectus excavatum and a subnormal IQ.

A more complex anomaly was described by Wang *et al.* (1962). A male child resembling by its malformations the trisomy 18 syndrome presented a double karyotypic anomaly: one of the chromosomes (13–15) was missing and one of the chromosomes 3 was submedian, one of its arms being longer than normal, while a ring chromosome was also observed.

These authors advance the hypothesis of a translocation to the chromosome 3 and ring formation of a 13 and 18 respectively, although it is not possible to specify the particular rearrangement undergone by each of them.

The same workers observed a second subject also male, in whom one of the (17–18) was replaced by a ring chromosome.

All theses cases producing by different mechanisms (translocations, insertions, ring chromosomes, supernumerary bearing a deletion, etc.) superfluous hereditary material indicate the possibility of using these "partial trisomies" to evaluate the gene content of chromosome 18 (cf. p. 132). This type of investigation proposed on several occasions (Patau *et al.*, 1961d) now runs up against two difficulties.

The first is of a clinical nature, the description of the symptoms being very often inadequate while the most typical cases of trisomies do not always involve all the known symptoms.

The second is of a logical nature and directly depends on the identification of the origin of the added fragment. In effect, the risk of circular reasoning may be difficult to avoid since apart from the case of a translocation transmitted by

one of the parents (Brodie and Dallaire, 1963) the identity of the fragment cannot be established cytologically. Under these conditions, it may be that a fragment of 18 is involved since certain symptoms are comparable with those of free trisomy 18 so leading to the conclusion that since this is a fragment of 18 the normal 18 contains genes controlling the symptoms observed.

(g) Other anomalies associated with trisomy 18

Four cases of association are known, two of which concern a sex anomaly: trisomy 18 and formula XXX (Uchida *et al.*, 1961a; Ricci and Borgatti, 1963); trisomy 18 and formula XXY (Haylock *et al.*, 1963b); and, finally a case of combined trisomy 18 and 21 (Gagnon *et al.*, 1961b).

In these four cases the stigmata of the two syndromes appear to be simply summated although the case of trisomy 18 + trisomy 21 resembles more closely trisomy 18.

Finally, in the same family (Hecht *et al.*, 1964) two affected sibs were observed, one girl with trisomy 21 and the other with trisomy 18.

The apparently too high frequency of these associations will be discussed later.

CHAPTER 6

Other Autosomal Anomalies due to Excess or Deficiencies

TOGETHER with the trisomies 21, 18 and 13 described in the previous chapters, many observations are steadily accumulating concerning much more varied morbid entities, classifications of which cannot at present be attempted.

At most, and for simplicity of exposition one can arrange the observations in relation to the morphological type of the supernumary element. It must be made clear that this aspect does not tell us anything *a priori* about the gene content of the surplus chromosome.

A. Small extra acrocentric

The Sturge–Weber syndrome characterized by a facial, cutaneous-mucous' "port-wine" naevus in the trigeminal region was related to a trisomy for chromosome 22 by Hayward and Bower (1960). These authors observed the presence of 47 chromosomes including a small extra acrocentric in a mentally defective child with this syndrome. However, many patients with this same syndrome are found to carry 46 normal chromosomes (Lehman and Forssman, 1960c, 2 cases; Institut de Progénèse, 1960, No. 333; Hayward and Bower, 1961, 7 cases; Gustavson and Hoch, 1961, 2 cases; Hall, 1961, 3 cases; and Patau *et al.*, 1961g, 2 cases).

Of all the observations on the Sturge–Weber syndrome only Patau *et al.* (1961) report a case with an abnormal karyotype. The anomaly consists of very small euchromatic arms (about one-third the size of a 21 chromosome) instead of and at the site of the filaments and satellites of a chromosome of type (13–15). These authors quite logically postulated the possibility of a partial trisomy for the translocated chromosome fragment which they liken by analogy with the observation of Hayward and Bower (1960) to a chromosome 22 fragment.

An ingenious piece of reasoning led them to consider all cases of Sturge–Weber syndrome with a normal karyotype to be partial trisomies masked by the imprecision of existing techniques. A duplication of 0·5 per cent of the length of the haploid genotype can only be detected in the special case of a translocation to the short arm of an acrocentric (compare Chapter 3). Such a generalization cannot, however, be directly accepted and absence of cytological proof cannot be considered as confirmation of a hypothesis.

Later, Dent *et al.* (1963a) re-examining the child described by Hayward and Bower (1960) concluded that the child had, in fact, an XYY karyotype.

Trisomy 22

Several cases of presumptive trisomy for a small acrocentric can be compared; Turner and Jennings (1961) report an 8-year-old boy with an almost normal morphology but with a receding chin, a flat occiput and enlarged nostrils, showing slight mental retardation with a marked schizophrenic tendency.

Dunn *et al.* (1961) described a boy with severe congenital hypotonia with oblique palpebral fissures, mental retardation and hypogonadism also bearing an extra small acrocentric chromosome. The same karyotype is reported by Zellweger and Mikano (1961a) in a mentally retarded hypotonic girl presenting a bilateral epicanthus.

The case reported by Koulischer and Périer (1962) is more complex. This was a child with multiple deformities who died at the age of 2½ months, also presenting a karyotype with 47 chromosomes including a small acrocentric. The malformations (heart defect, bronchial peri-arterial sclerosis, thinning of the cranial vault and generalized hypotonia) and the appearance of the child did not suggest trisomy 21, according to the authors. The mother of the child presented a mosaic, cells with 46 and cells with 47 chromosomes.

In all these cases diagnosis of trisomy 21 was rejected on the grounds of the clinical pictures and the problem of existence of a true "trisomy 22" is thus raised. However, because of the lack of definition of the clinical picture it is premature to define such an entity.

Examples of mosaicism for an extra acrocentric as in the case of the mother mentioned by Koulischer and Périer (1962) have been reported. Biesele *et al.* (1962) and Schmid *et al.* (1962c) have described a couple of very slender, monozygotic twins, mentally retarded and with a marked schizoid tendency, without clinical signs of trisomy 21; both presented a mosaic of cells some normal with 46 chromosomes, the others with 47 showing an extra small acrocentric.

The same type of mosaicism is reported by Siebner *et al.* (1963) in two sibs with Pelger–Huet anomaly (granulocytes with rounded nuclei simulating mononuclears).

It should be noted that apart from these observations many cases of severe congenital hypotonia, schizoid state and the Pelger anomaly present normal karyotypes.

The absence of a strong correlation between clinical symptoms and karyotypic anomalies means that these observations must await classification. Only accumulation of new data will enable us to judge their significance.

B. Giant satellites

The question of "giant satellites", that is, displaying a development considerably greater than normal, has been raised in several disorders.

Arachnodactyly or Marfan's dolichostenomely, a dominant genetic disease, has given rise to much discussion. Tjio *et al.* (1959/1960) were the first to report the existence of a particularly large satellite in two sporadic cases of the disease:

one a giant satellite on a (13–15) and one on a (21–22) in the second case. Many failures to detect such giants in several laboratories including ours make any possible causal relation between this feature and Marfan's syndrome extremely doubtful (McKusick, 1960; Handmaker, 1963).

Kallen and Levan (1962) consider that the relative length of the four chromosomes (21 + 22) was a little shorter in the 4 patients studied by them than in 10 normal subjects with whom they were compared. Despite the difficulties of such analysis, these authors postulated a possible genetic control of the length of the chromosomes.

Ellis and Penrose (1961a) describe a family in which some members died with malformations of the central nervous system; in two normal surviving subjects they established existence of a giant satellite on chromosome 13.

Cooper and Hirschhorn (1961a) and Cooper (1962) mention two families in which a giant satellite was found in several subjects. The first family included a child whose morphology resembled that of trisomy 21, with epicanthus, slit eyes, flat bridge of the nose, unilateral transverse palmar crease and mental retardation. This child, with 46 chromosomes, showed very prominent satellites on one of the small acrocentric chromosomes 22. The father and two other children bore the same trait, while the mother and a fourth child had normal satellites.

The second family was characterized by the presence of an exceptional trisomy 21 by translocation 21 ~ 21 (cf. Chapter 7) without this translocation being found in the parents or the sibs. This child, with acute lymphoblastic leukaemia, displayed a "giant satellite" on one of the large acrocentrics, the 13 (?). This same giant satellite was found in the father and two sisters of the propositus while neither his mother nor his brother possessed it.

These authors attribute the first observation to a possible translocation, the father and two of his children carrying a balanced translocation, the abnormal subject carrying the translocation in a combination leading to the presence in triplicate of the translocated segment.

For the second family the problem is that of knowing whether the karyotypic feature of the father played a role in the appearance of the 21 ~ 21 translocation in his son.

This question of "giant satellites" takes us back to what was said in Chapter 3 on the variability of these structures.

The absence of detectable morphological or constitutional disturbances in subjects with balanced translocations (with loss of one or two satellites) indicates that this material has little genetic activity. It thus becomes difficult to relate an augmentation to a pathological effect. Furthermore, the syndromes presented for carriers of giant satellites are highly variable as is demonstrated by two cases observed in our laboratory. One a right-hearted hypogonadic hypospade (Institut de Progénèse, No. 229) may be interpreted as a 21 ~ Y translocation, the other (Institut de Progénèse, No. 121) with very severe hypospadias regularly presented a duplication of the satellites of one of the chromosomes 21 (cf. Fig. 6.1).

However, Miller *et al.* (1962d) noted the presence of a large satellite of

chromosome 13 in two sisters in other respects normal and de la Chapelle *et al.* (1963a) observed normal familial segregation of a chromosome 21 with large satellites.

It is extremely difficult to judge these observations on "giant satellites" and before establishing the relation between the cytological feature and a given clinical anomaly it should be remembered that the extreme heterozygoty of

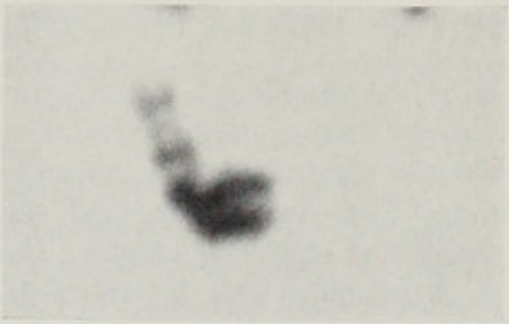


FIG. 6.1. Duplication of satellites on chromosome 21 in a patient with hypospadias (Institut de Progénèse, No. 229).

human populations may in itself account for these phenomena. It is known in fact, that crepis studied by Nawaschin (1927) exhibited the curious phenomenon described as "amphiplasty" by this author. The satellites of crepis may appear or disappear according to the origin of the parents of the hybrid by retraction of the filament which links them to the chromosome body. Appropriate crossings enabled Nawaschin (1934) to demonstrate that the satellites are not, in fact, lost since they can reappear as a function of the genome. More generally, certain varieties are thought to have a greater affinity for nucleolar material and to utilize it completely so that the homologues derived from less vigorous varieties from this point of view can no longer form satellites. The cytological analogy of the satellites of human acrocentrics and those of crepis suggests the existence of a phenomenon of this nature in the appearance of satellites, particularly since the association between the acrocentrics of man and nucleolar material appears to be well established (Ohno *et al.*, 1961d).

C. Extra or abnormal medium-sized chromosome

The individual diagnosis of an extra medium-sized chromosome is particularly intricate (cf. Chapter 3). In all the cases mentioned below the possibility of an extra X can only be excluded in the absence of excess chromatin bodies. Such exclusion, however reasonable it may be, cannot be formal and at any rate, it in no way solves the problem of the actual identity of the supernumary.

Butler *et al.* (1962) describe a boy born before term who died at 8 weeks with pyloric stenosis, complete agenesia of the left lung and Hirschsprung's disease. The extra chromosome seems to have been a medium-sized chromosome approximately of the size of a 6.

A slightly deformed negro child with incomplete sexual development (hypogenitalism) was described by El Alfi *et al.* (1963) as presenting a mosaic of cells with 46 chromosomes (normal XY karyotype) and cells with 47 chromosomes (a medium-sized chromosome in excess). Although rejected by these workers, the hypothesis of an XY/XXY mosaic is quite plausible here. On the other hand, absence of genital disturbances and the presence of anomalies of the face (low set ears) and of the skeleton, renders less probable the hypothesis of an XY/XXY mosaic in the young boy described by Pfeiffer *et al.* (1962e) although the presence of hypospadias and cryptorchidism suggests this explanation. The feeble-minded but well-formed boy described by Stadler *et al.* (1963) showed a cytologically similar 46/47 mosaic. The absence of a chromatin body and sex disturbances also led the authors to reject the hypothesis of an XY/XXY mosaic, which is, however, compatible with the purely morphological findings.

Likewise, the case of a 59-year-old woman (reported by Lewis *et al.*, 1963), dwarfish, hypogonadic and mentally retarded, and described as having trisomy 16, may be likened to sex aneuploidies with anomalies of structure of the X chromosome, as can the following case.

An extra medium-sized chromosome coupled with absence of a chromosome 16 was reported by Jennings and Turner (1961) in a mentally retarded girl not having reached puberty at the age of 14 years with epicanthus, cleft pelate and webbed neck.

The same authors (Turner *et al.* [1962]) reported a case of a medium-sized ring chromosome in a mentally-retarded hirsutic subject with loss of this ring in a certain number of cells (resulting in 45 chromosomes).

Bray and Mukherjee (1963) describe a mental defective characterized by a rearrangement of chromosome 16.

Apart from these relatively definite syndromes, a family showing a chromosome variation was reported by Böök *et al.* (1961b); a boy and his mother had interauricular communications. The child had 46 chromosomes with an apparent trisomy (19–20) and monosomy 22, or a (19–20) ~ 22 translocation while the mother presented a mosaic of normal cells (20 per cent) and cells with 47 chromosomes (80 per cent) with apparent trisomy (19–20). A sister of the mother with the same interauricular communication presented a majority of cells with 47 chromosomes with apparent trisomy for a medium-sized chromosome of group 6–12. Finally, a normal girl belonging to the same family presented an apparent heterozygous 2 ~ (6–12) translocation. Such chromosome variation suggests the existence of mutator genes in such an exceptional family.

D. Hyperploidies

The existence of three instead of two genomes has been reported several times giving triploid cells. Experimentally, triploidy appears to be incompatible with embryonic develoment, at least in mammals (Piko and Bomsel-Helmreich, 1960), while it is perfectly compatible with life in *Drosophila*.

The first case of triploidy in man (Böök and Santesson, 1960c) consisted of a

cellular mosaic some diploid and the others triploid in cultures of skin and fascia while the bone marrow cultures revealed exclusively diploid cells (Böök, 1961a). The child with porencephaly with serious mental deficiency showed micrognathia and syndactyly of both fingers and toes.

It seems probable that the embryonic development of this child was rendered possible by the large number of diploid cells. The same phenomenon of a diploid/triploid mosaic was observed by Ellis *et al.* (1963b) in a mentally retarded girl of quite normal appearance. Analysis of the blood groups enabled these authors to consider the triploid cells as being of the genotype 00B.

On the other hand, pure cultures of triploid cells have been obtained in spontaneous abortions: intact embryo at 2·5 months (Delhanty *et al.*, 1961); macerated 5-month foetus (Penrose and Delhanty, 1961c); and in four abortions of a total of 82 studied (Carr, 1963a).

These few examples again reveal the stringent selection which operates *in utero* against chromosome anomalies.

Recent statistics of Carr (1963b) refer to 50 cases of abortion or foetal death among which the author lists 12 constitutional chromosome anomalies:

haplo X karyotype	3 cases
trisomy 13	3 cases
trisomy 18	2 cases
trisomy 21	1 case
triploidy	2 cases
tetraploidy	1 case

This sample, the only one available, is of extreme interest in that it reveals the very important role of chromosome anomalies in spontaneous abortions and foetal deaths.

From the figure of 12 in 50 it would appear that almost one foetal death in four is directly determined by an anomaly in chromosome segregation. If one considers that only flagrant changes can be detected at the present time, it is reasonable to conclude that chromosome aberrations represent one of the most important factors in the arrest of foetal development. On the other hand, hydatiform mole does not seem, according to Sasaki *et al.* (1962a), to be accompanied by specific chromosome anomalies.

E. Autosomal deletions

Apart from the slight deletions brought about during translocation by centromeric fusion (Chapter 7) two types of isolated deletion, without translocation, are now known.

Deletion of the short arm of 18

Grouchy *et al.* (1963j) described a particular dysmorphic syndrome in a boy with severe mental retardation. They noted the following signs: hypertelorism with incomplete convergence in the right eye, low set ears and deformed hands (high

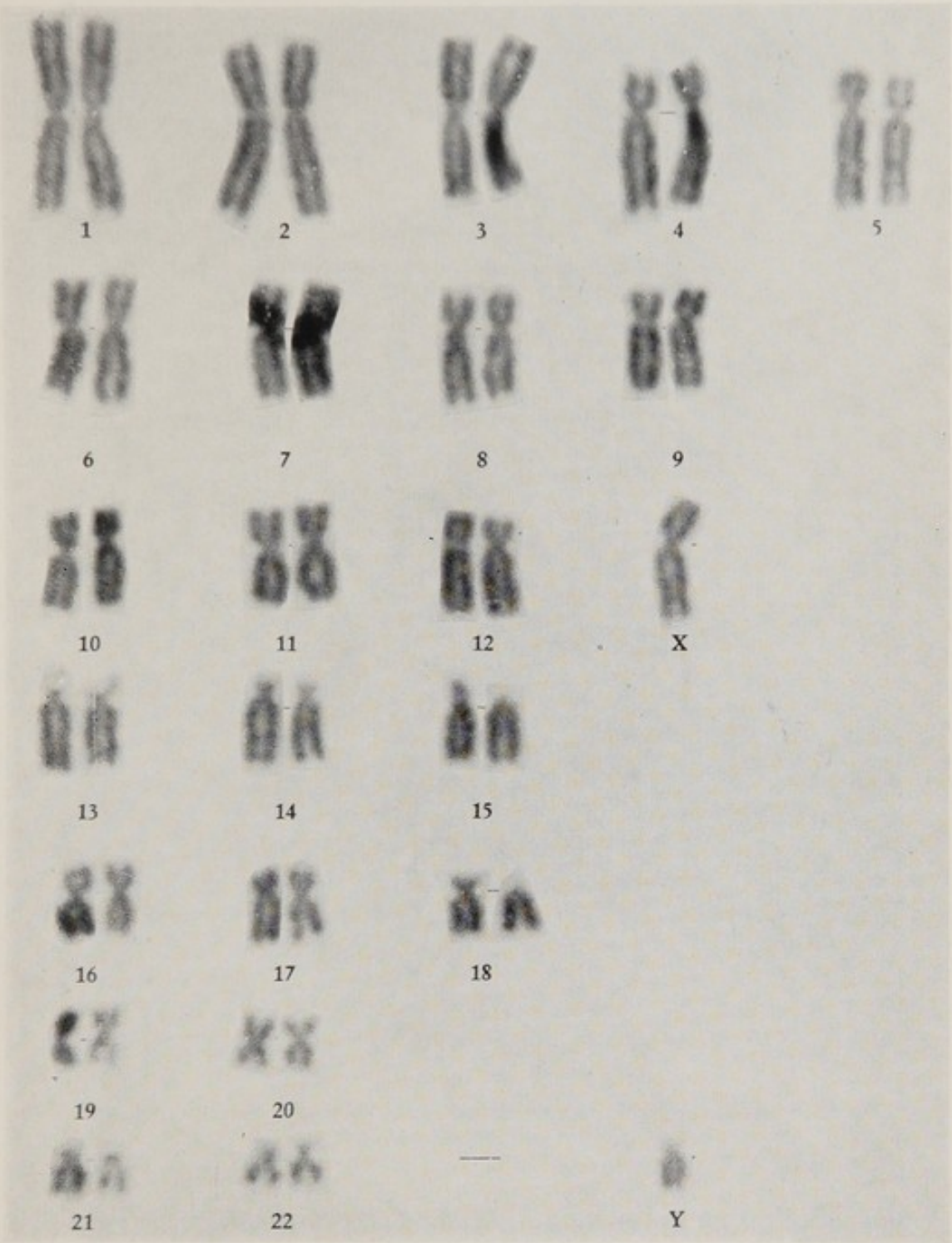


FIG. 6.2. Karyotype of a boy with multiple malformations. Absence of short arm of chromosome 18. (Photography by courtesy of Dr. de Grouchy.)

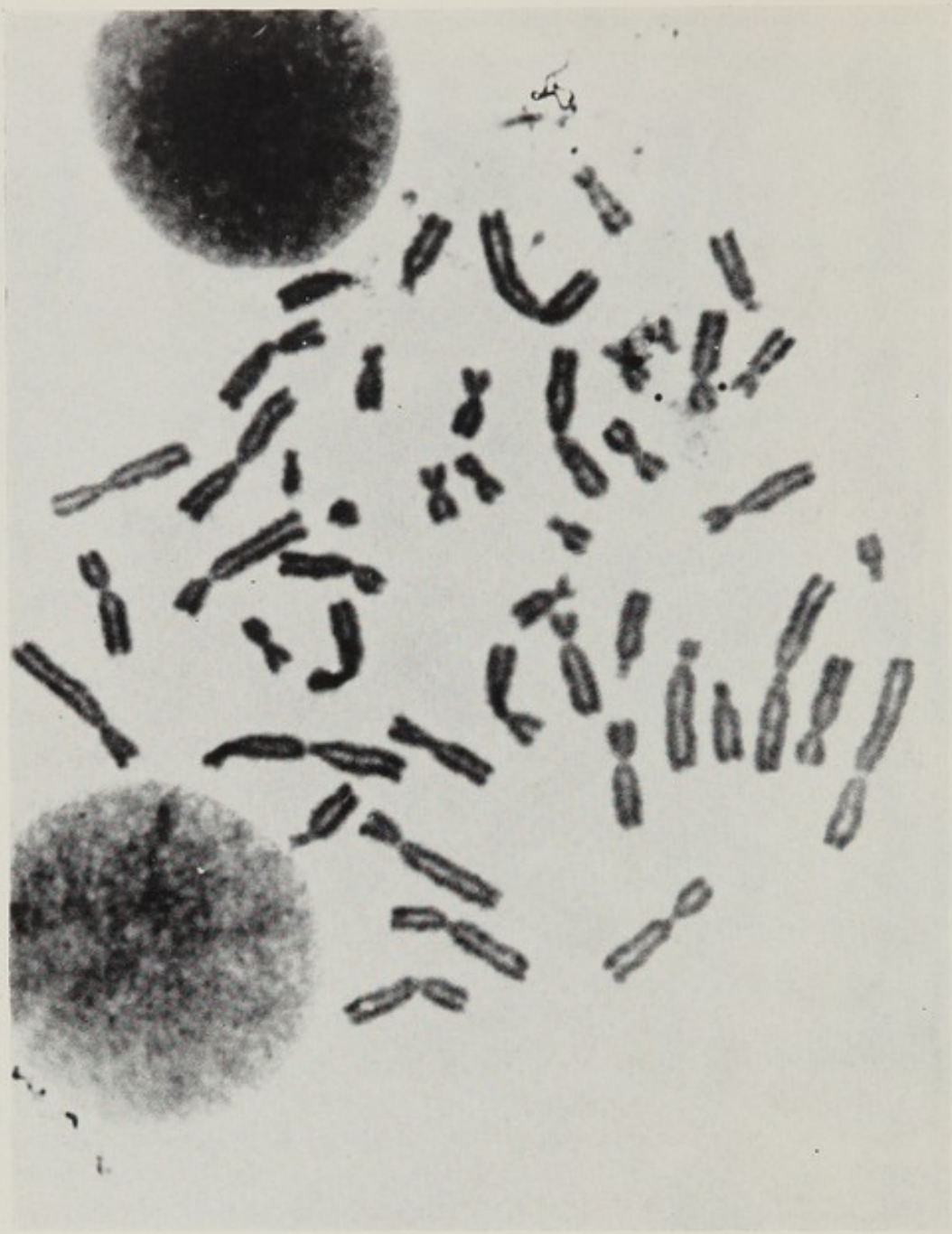


FIG. 6.3. Cell of a boy with partial deletion of the short arm of a chromosome 5 (Institut de Progénèse, No. 881).

setting of the thumb and curving of the fifth finger) and feet (syndactyly of the third and fourth toes). The karyotype with 46 chromosomes revealed the absence of a chromosome 18 replaced by a telocentric the arm of which was identical in size to that of the long arm of 18 (Fig. 6.2). The most plausible explanation for such an anomaly is loss of the short arm of 18 just above the centromere.

A very similar case with palpebral ptosis, low set ears, micrognathia and

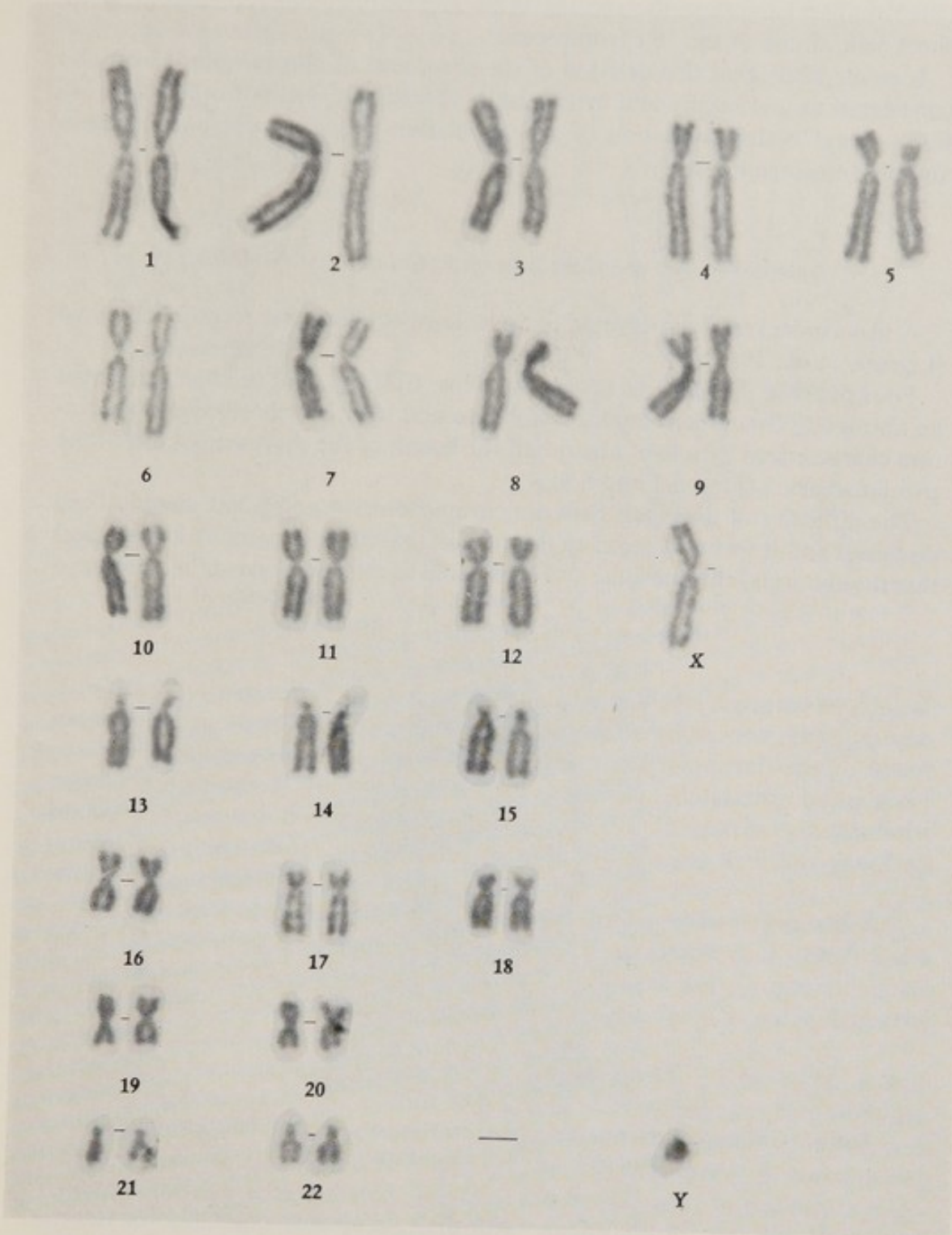


FIG. 6.4. Karyotype of the same boy with partial deletion of the short arm of chromosome 5 (Institut de Progénèse, No. 881).

anomalous articulation of the thumb is briefly mentioned by Lewis *et al.* (1963b). The karyotype showed the same anomaly, complete deletion of the short arm of one of the 18 chromosomes.

It seems likely that this deletion of the short arm of chromosome 18 may be considered as a clinically and cytologically identifiable entity if other observations permit better evaluation of the correlation between the clinical picture and chromosome anomaly.

Deletion of half the short arm of 5 (Cri du chat syndrome)

A new clinical entity attributed to an isolated deletion was recently observed (Lejeune *et al.*, 1963b).

Four patients (Institut de Progénèse, Nos. 676, 881, 869 and 968) presented an absolutely concordant clinical syndrome and identical chromosome aberration characterized by loss of about half the length of the short arm of one of the chromosomes 5 (Figs. 6.3 and 6.4).

The difficulty of diagnosis between chromosomes 4 and 5 has already been discussed and it was only from an impression (based on very many karyotypes) that the abnormal chromosome was considered as replacing one of the 5 chromo-

FIG. 6.5. Girl with partial deletion of the short arm of a chromosome 5 (Institut de Progénèse, No. 676). ("Cri du chat" disease.)

somes. At any rate, in accordance with the rule already proposed for the trisomies 21, 18 and 13, we suggest that the number 5 be definitively given to the chromosome, partial deletion of the short arm of which determines the clinical syndrome observed which we can briefly describe as follows: multiple malformations including microcephaly, hypertelorism with epicanthus, normal ear lobes but low set; and micro and retrognathism (Fig. 6.5).

The dermatoglyphs reveal three features: axial triradius in position *t'*, transverse palmar crease (or equivalent) and absence (or vertical position) of the triradius of the fourth finger (Fig. 6.6).

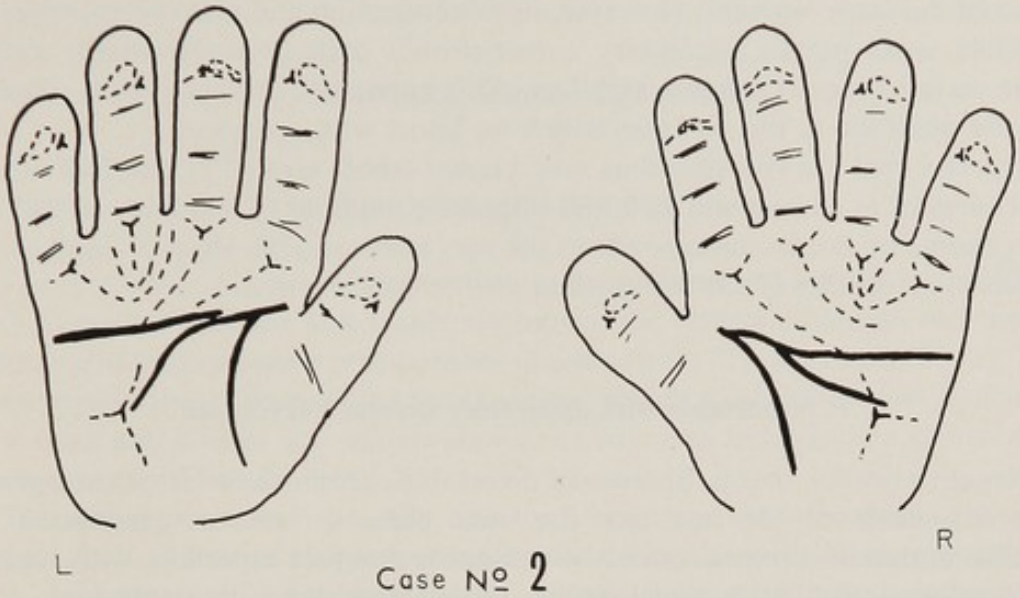


FIG. 6.6. Dermatoglyphics of a child with partial deletion of the short arm of a chromosome 5 (Institut de Progénèse, No. 881).

In the first three cases, no cardiac involvement was demonstrated by clinical, radiological and ECG examination; the fourth presented an Ivemark syndrome.

General involvement: Considerable hypotrophy in stature and weight. Severe mental deficiency. A particular consonance of crying simulating by its sharp timbre and plaintive tonality the miaowing of the cat. This latter sign, extremely typical in the first month of life, seems to be related to a supraglottal hypoplasia with laryngomalacia.

To these four cases has been added a quite similar observation which Professor Böök was kind enough to communicate to us (Böök *et al.*, 1963) and a sixth observed by Grouchy *et al.* (1964a). The clinical and cytogenetic agreement of these cases allows this disorder to be considered as a distinct entity (Lejeune *et al.*, 1964).

Recently, familial segregation of a translocation 5 ~ 13 provided genetic evidence that the *cri du chat* syndrome is actually determined by monosomy for a fragment of the short arm of 5; the reciprocal syndrome, trisomy for the same fragment has also been observed (Lejeune *et al.*, 1964a). Finally, in two separate patients labelling with tritiated thymidine inculpated pair 5 in both cases (German *et al.*, 1964, personal communication).

A chromosome lesion quite comparable with that affecting these six patients was mentioned by Hirschhorn and Cooper (1961) in a child presenting a central defect of the scalp, a right iridocoloboma, absence of septum pellucidium, a fissure of the soft palate and a hypospadias. At present, it is difficult to say whether this case lies within the framework of the syndrome described above

or whether it should be considered as an anomaly affecting another chromosome, 4, for example.

It is possible that the clinical picture is more complex the larger the fragment lost and there is no formal reason for postulating that the deletion must always concern the same segment. However, natural selection and also the existence of possible weak points (secondary constriction?) perhaps preferentially determine certain types of deletion and, hence, the apparent coherence of the clinicial picture observed in the six cases which we know with precision.

The fact that the two deletions now known (short arm of 18 and half of the short arm of 5) correspond to a loss of genetic material of less than 1/100th of the genome no doubt corresponds to the very unfavourable effect of haplosomy which in all species is not tolerated as well as hypersomy.

F. Syndromes with apparently normal karyotypes

Negative results, that is, absence of detectable chromosome lesions in a given disorder, obviously do not have the same didactic value as description of specific anomalies. Imperfections of present techniques especially with regard to translocations (Turpin and Lejeune, 1961c) may always be implicated.

Harnden (1961) attempted a review of malformation syndromes in which the karyotype is apparently normal. We shall only mention here the most striking among the large number of syndromes studied.

Typus degenerativus amstelodamensis (de Lange's syndrome), despite a stereotyped aspect, does not involve detectable anomalies according to Hienz (1963), Laurence and Ishmael (1963) and various other laboratories.

Nor does supravalvular aortic stenosis which involves a very characteristic facies (Eberle and Beuren, 1963; Joseph *et al.*, 1963; Grouchy and Emerit, 1963d) although Palmer (1963) reports a case of 46/47 mosaicism through presence of a small extra metacentric (19 or 20). Twelve other patients reported by this author had a normal karyotype.

Böök *et al.* (1961) reported four cases of Laurence–Moon–Biedl syndrome, all with normal karyotype, and the same absence of chromosome disorder is reported by Chu *et al.* (1961) and Fraccaro *et al.* (1961) in the syndrome improperly called male Turner's syndrome.

We would mention for the record the normal karyotypes reported in cases of the syndromes of Werner (Fraccaro *et al.*, 1963; Motulsky, 1962), Cockayne (Windmiller, 1963) and Hurler (Rosenthal, 1961; Gayler and Fried, 1962) and in pseudohypoparathyroidism (Schleich *et al.*, 1963).

Finally, two reports refer to uncertain karyotypes (probably normal) in cases of "convulsions" and "cerebral palsy" (Dobson and Ohnuki, 1961; Blumel *et al.*, 1961 b.).

CHAPTER 7

Structural Rearrangements

TRANSLOCATIONS

The first observation of translocation in man concerned a hypotrophic child with mental retardation and malformations of the vertebral column and hence, the name *polydysspondylie* proposed for this condition (Turpin *et al.*, 1959a). The karyotype of this child revealed the existence of only 45 chromosomes by fusion of a small and a large acrocentric giving rise to a new hybrid quite similar to a chromosome 10 or 12.

This observation was the only one for some time until the publication by Polani *et al.* (1960) of the first exceptional trisomy 21 with 46 chromosomes. The child presented a translocation of a small acrocentric onto a large acrocentric and this rearrangement produced a masked trisomy.

Since the number of reported translocations increases every day it is now possible to attempt a morphological classification of these aberrations.

The most frequent, at least in our present state of knowledge, concerns two acrocentric chromosomes which fuse at the level of their centromere to form a new hybrid chromosome. This element is mediocentric if the two partners are of the same sizes or submetacentric for a small acrocentric (21–22) translocated onto a large acrocentric (13–15).

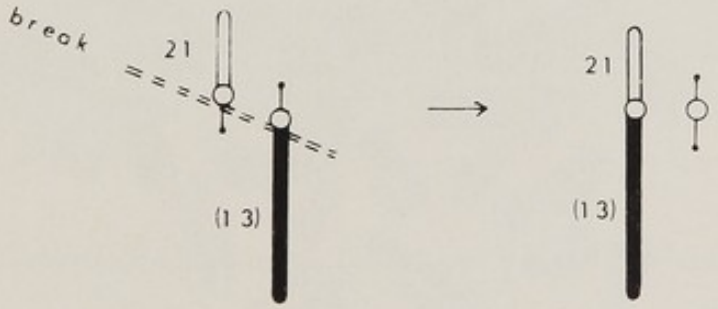


FIG. 7.1. Scheme of the mechanism of D ~ G translocation. (Centric fusion.) The centric fragment is secondarily lost.

The general process depicted in the diagram in Fig. 7.1 is currently termed centric or centromeric fusion. This is abuse of language for, as shown by the diagram, this type of rearrangement implies the existence of two chromosome breaks close to the centromeres and elimination of one centromere with the short arms attached to it.

However, it should be noted that the great majority of "centric fusions" do not involve any visible disadvantage for the heterozygous carriers. This fact, coupled with the observations of familial transmission over many generations, has led to the notion of chromosome polymorphism in our species (Lejeune *et al.*, 1960) somewhat similar to that described by Ford *et al.* (1957) in the common shrew.

The phenotypic effects of some of these translocations are very probably determined by minor deletions which may affect either the eliminated short arm or a paracentric segment of the remaining long arm. The use of these probable though not detectable deletions has been proposed for the purpose of identifying the position of certain genes (Turpin and Lejeune, 1961c).

Along with these translocations between two acrocentrics there are others between one acrocentric and another chromosome as well as translocations between two metacentric chromosomes.

To simplify the presentation we shall therefore review translocations between acrocentrics (between small and large, small and small and large and large), then the other types of rearrangement now known.

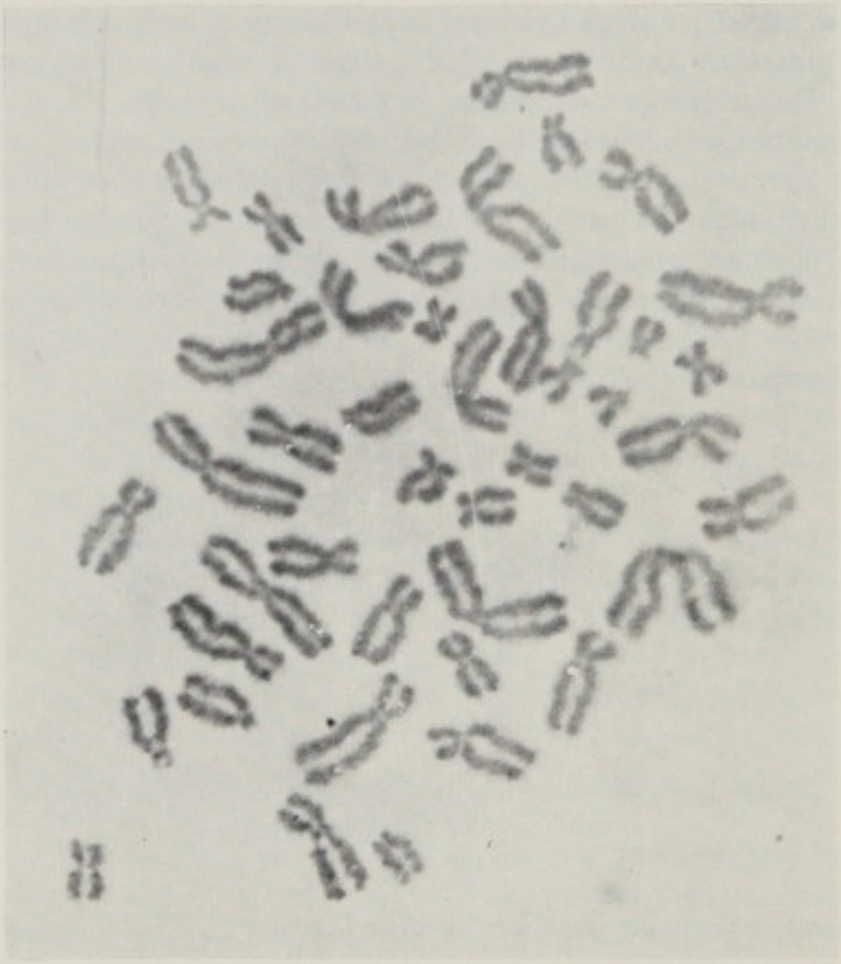


FIG. 7.2. Cell of a child with polydysspondyly.

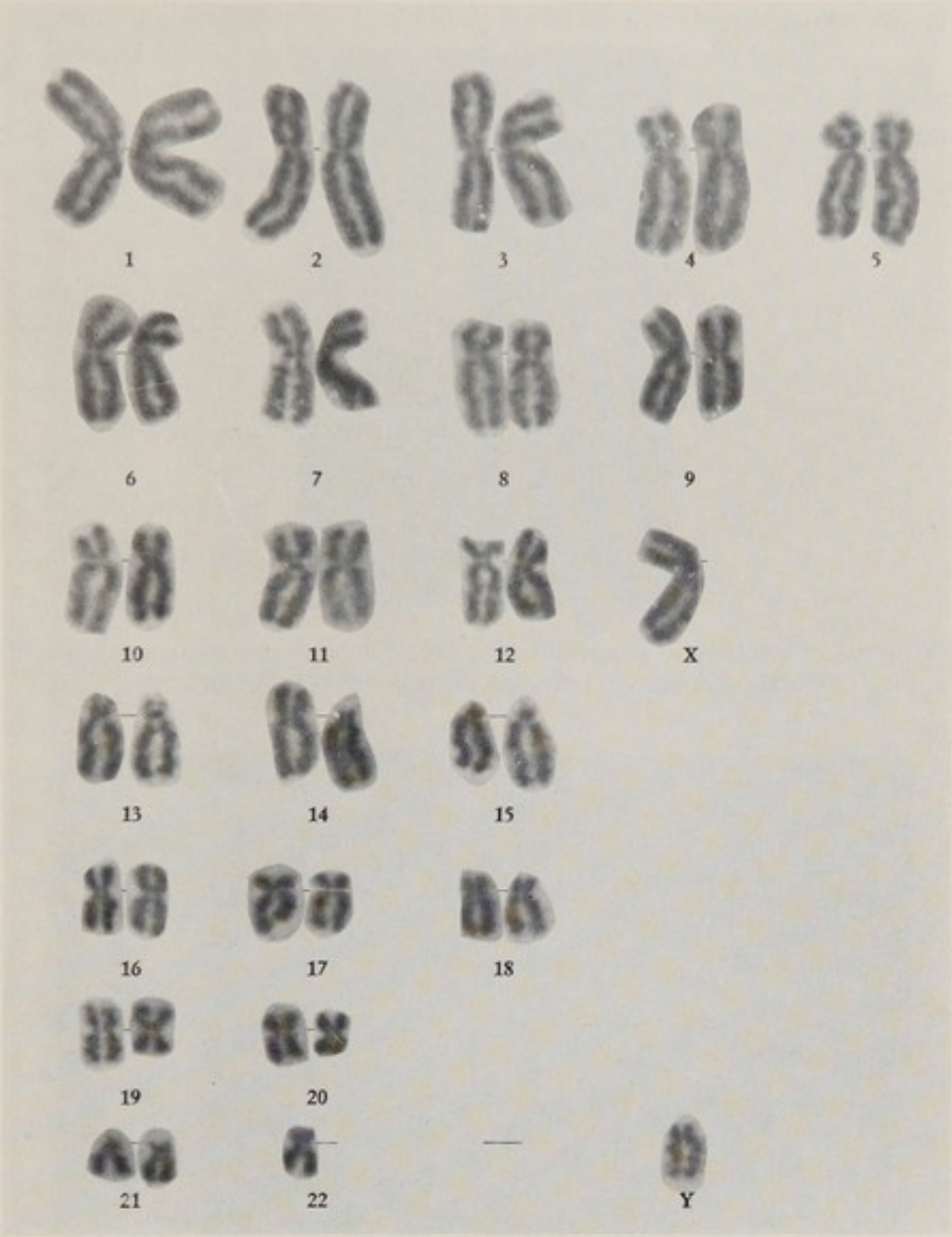


FIG. 7.3. Karyotype of a child with polydysspondyly (G ~ D translocation).

1. Translocations between a small and a large acrocentric

Type G ~ D

The individual diagnosis of acrocentric chromosomes is highly uncertain and it will be appreciated that the "soldering" of two of them can only complicate the problem because of competition between the acrocentries for the nucleolar material apparently composing the satellites.

The need for a simplified system of writing led us in 1959 to propose a classification by group, the small v-shaped acrocentrics being symbolized by the letter v, the large acrocentrics by the letter T (telocentric). A second classifica-

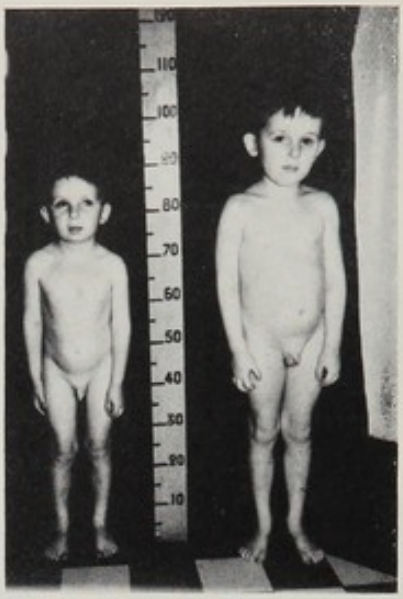


FIG. 7.4a. Comparative development of a feeble-minded polydysspondylic child with G ~ D translocation and normal child of the same age.

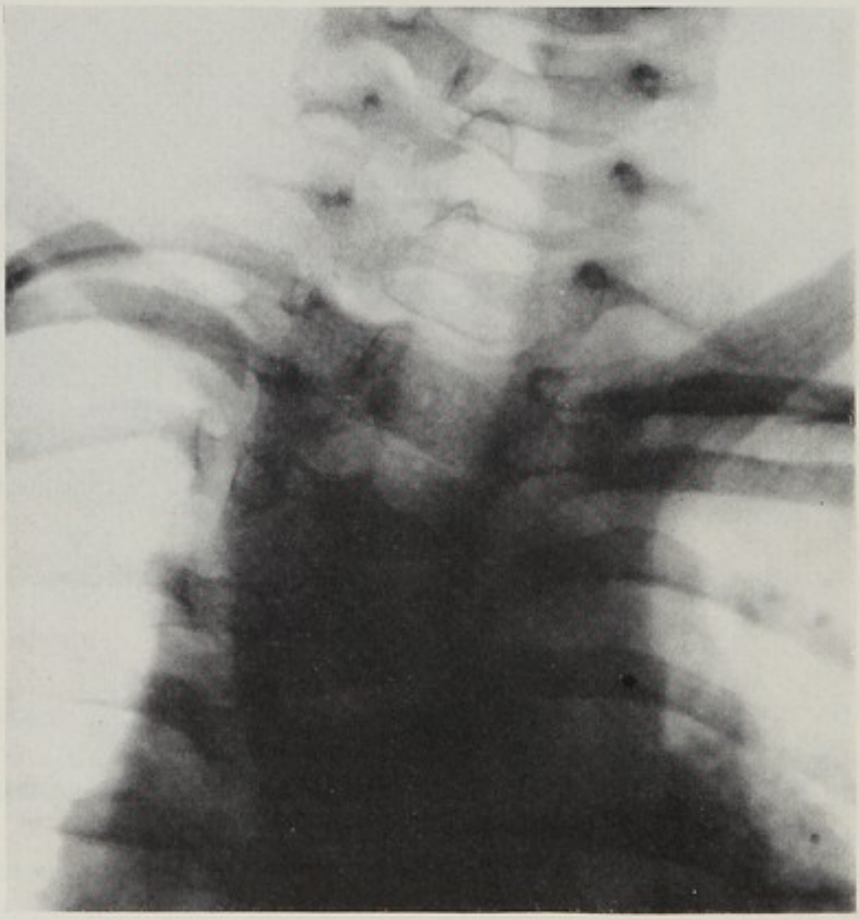


FIG. 7.4b. Frontal radiograph of the polydysspondyly.

tion by lettering was proposed by Patau (1960a) whereby the small acrocentrics are designated by the letter G and the large ones by the letter D.

Since we are dealing here with a mere convention whose sole purpose is to express clearly the observational evidence we employ the group D and group G classification which is more generally used.

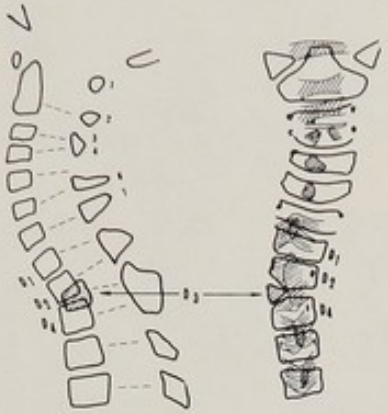


FIG. 7.4c. Diagram of the spine in polydysspondyly.

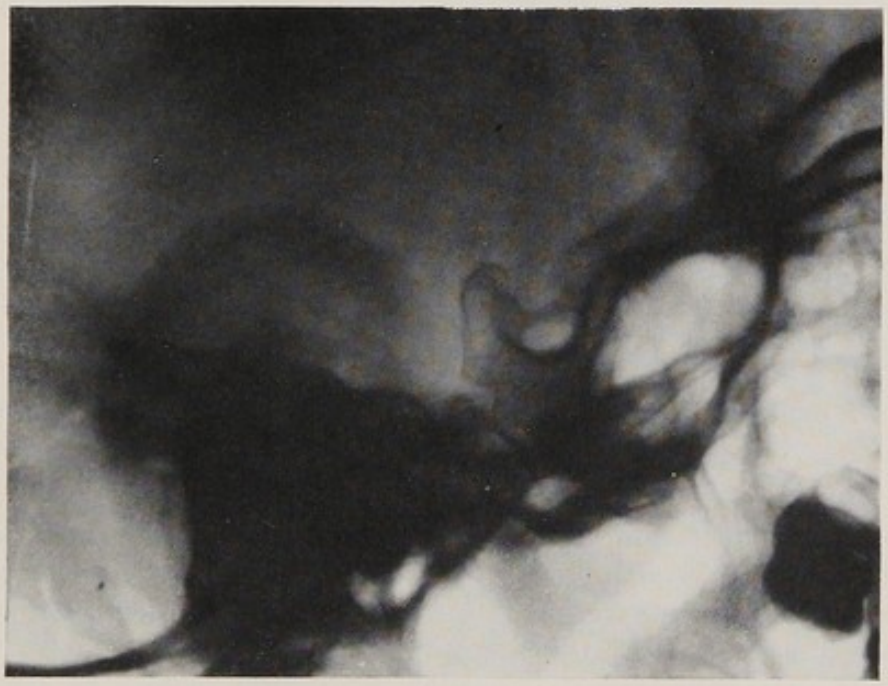


FIG. 7.4d. Radiograph of the sella turcica showing calcification of the interclinoid ligament.

Under these conditions, G ~ D covers six types of translocation which we cannot at present distinguish from each other and which are:

21 ~ 13	22 ~ 13
21 ~ 14	22 ~ 14
21 ~ 15	22 ~ 15

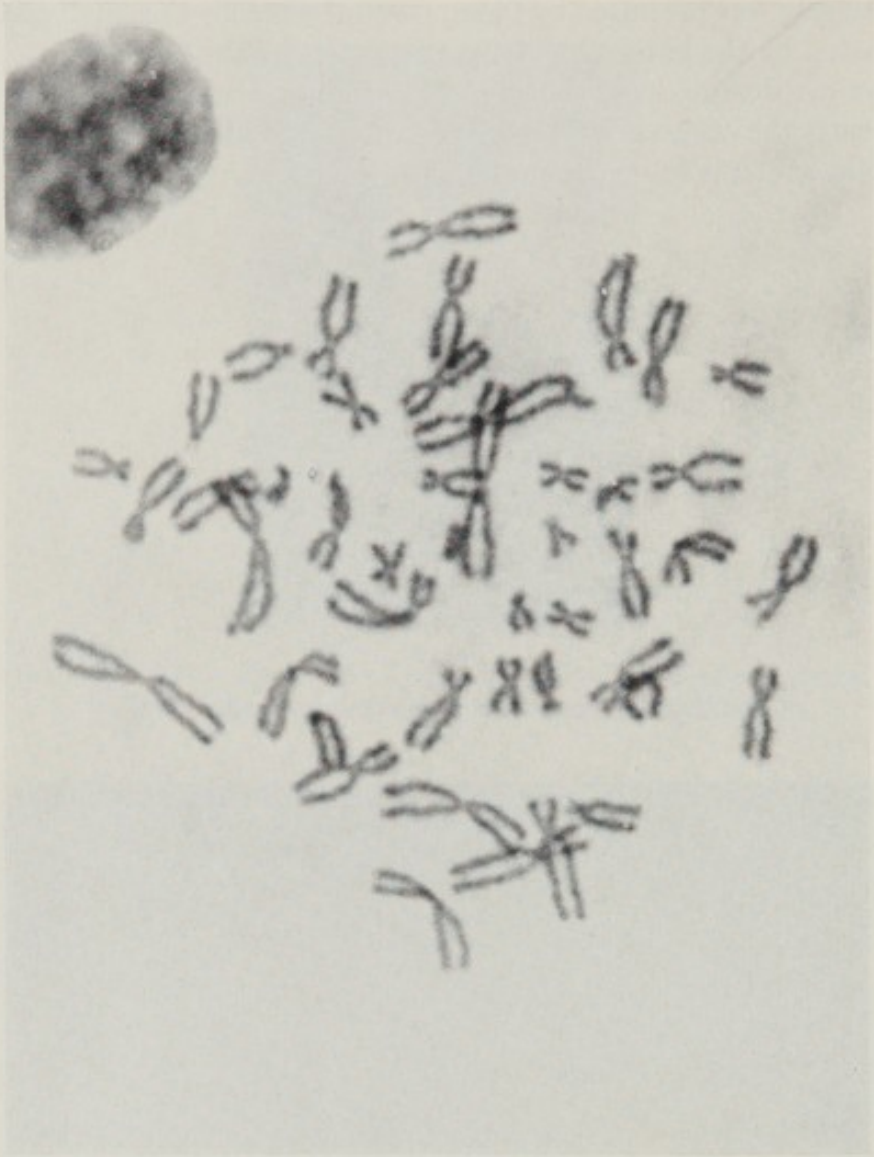


FIG. 7.5. Cell of an XXY subject with a 22 ~ D translocation (Institut de Progénèse, No. 293).

This simple enumeration shows that the term "G ~ D" must be abandoned and replaced by the designation appropriate to each case as soon as cytogenetic progress permits. In the meantime, this term must be considered as a simple expression of a morphological observation.

However, in certain cases it is possible by genetic analysis to deduce the origin of one of the chromosomes implicated in the rearrangement and it becomes possible to individualize among all the translocations G ~ D, the 21 ~ D which is present in certain families.

(a) Type G ~ D

The child whose condition we reported in 1959 (Turpin, Lejeune, Lafourcald and Gautier) was 4 years 5 months old. The salient features of his condition

were his underdeveloped stature and corresponding low weight (0·92 m and 12·200 kg) (Fig. 7.4a), retarded mental awakening (IQ = 89) and very considerable speech difficulties. In addition, examination of the spine revealed numerous anomalies which justified the description "polydysspondylie": narrowing of C_3–C_4 with posterior block; rachischisis of C_5; narrowing of T_1–T_2 and T_3–T_4 with posterior block; reduction in T_3 in the right hemivertebra; presence of the third rib on the right, not present on the left; right hemilumbarization of S_1; spatulate deformation of the 9th, 10th and 11th ribs (Fig. 7.4b,c). This polydysspondyly was accompanied in the sella turcica by calcification of the interclinoid ligament (Fig. 7.4d). A right hypothenar ulnar loop

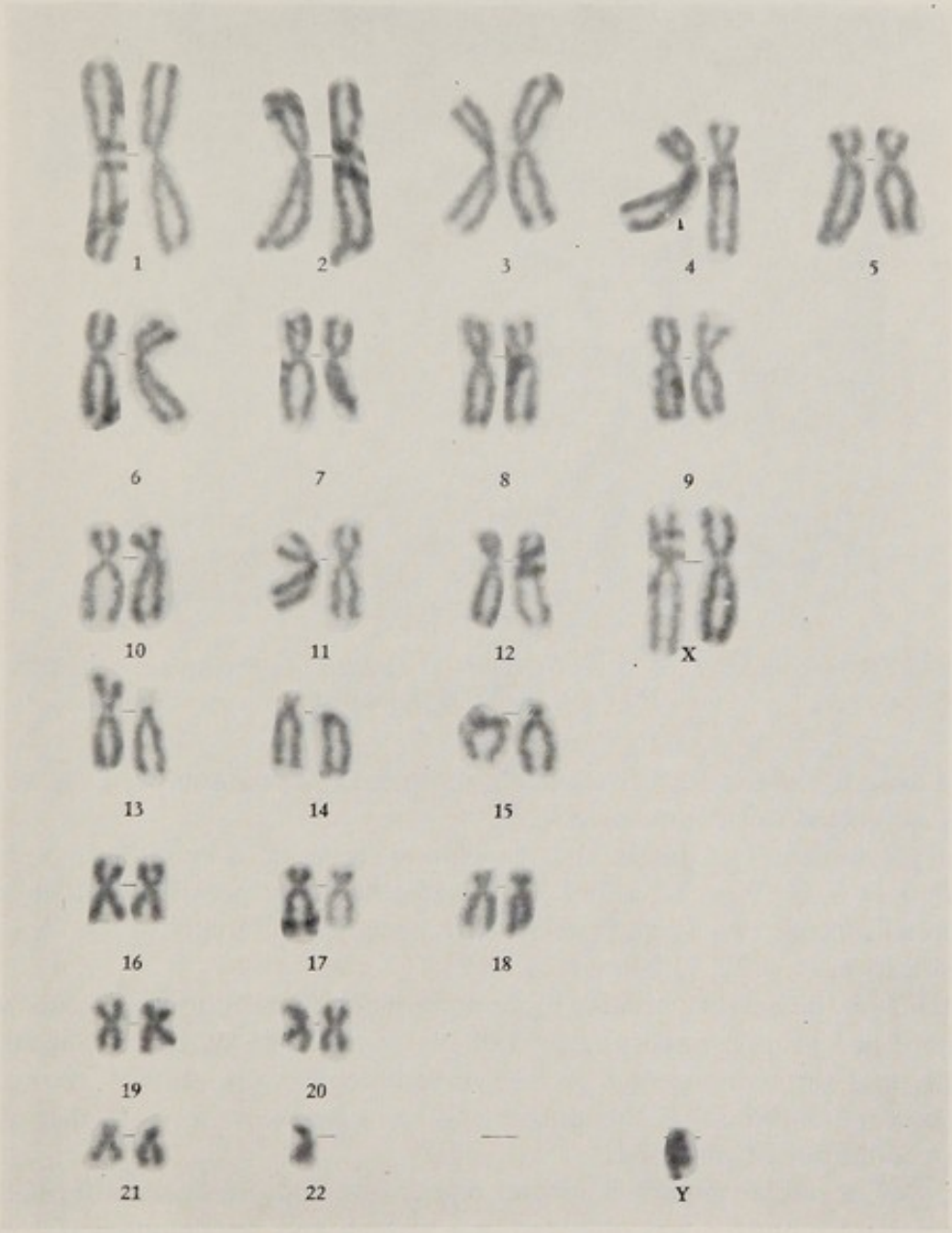


FIG. 7.6. Karyotype of an XXY subject with 22 ~ D translocation.

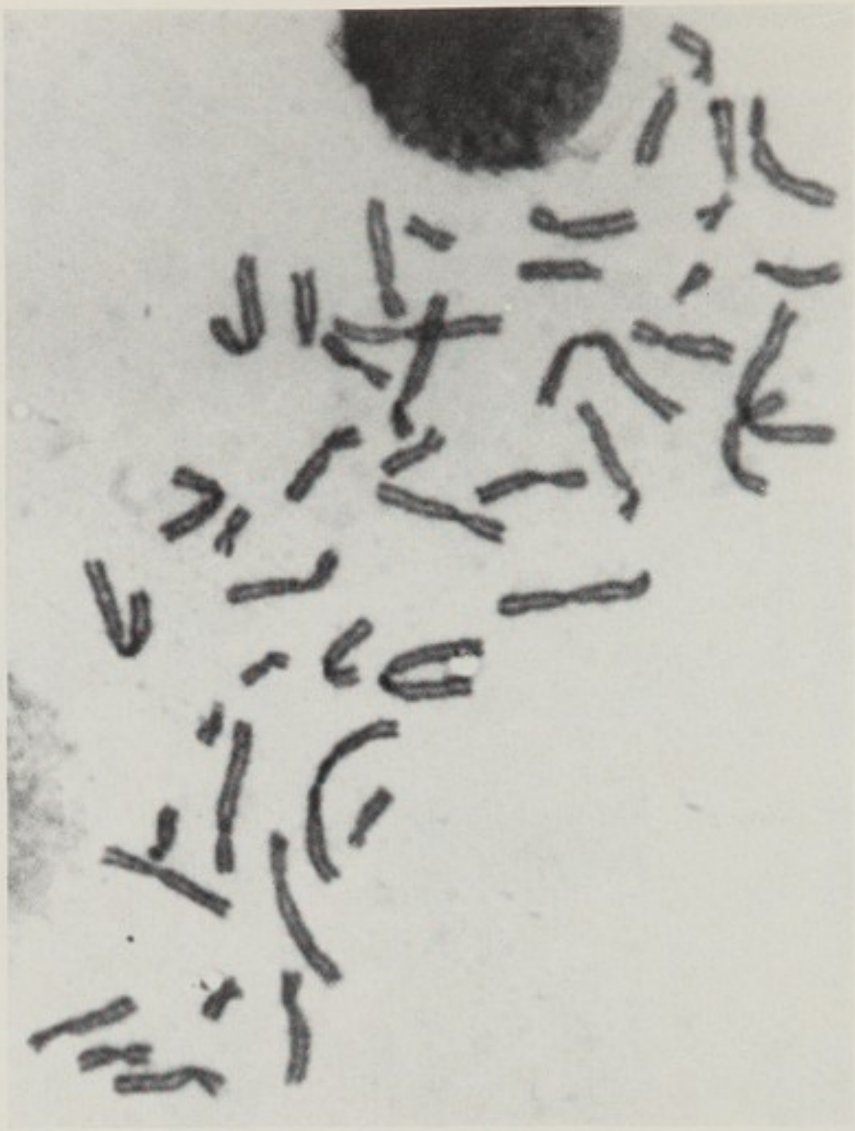


FIG. 7.7. Mother of a child with trisomy 21 (Institut de Progénèse, No. 1071). Note the 21 ~ D translocation.

and a lowering of the nuclear segmentation index of the granulocytes (2·37 and 2·62) completed this constellation.

Karyotypic analysis (fascia and then blood) revealed a heterozygous translocation D ~ G (Figs. 7.2 and 7.3). The identification possible by their conventional signs of two 13 and two 21 chromosomes led finally to the idea of a translocation D ~ 22, D being a 14 or 15.

With age, the debility became more accentuated. The following values were obtained at 8 years 3 weeks: height 1·08 m, weight 15·95 kg, IQ 72. The father, mother and the two sisters were free of radiological and clinical anomalies. The karyotypes (blood) of the mother and one sister were normal—that of the father could not be obtained.

In 1963, a similar picture of mental retardation and physical malformations with polydysspondyly was observed in a woman and her daughter (Grouchy *et al.*, 1963). A D ~ D translocation was found in these two subjects (see p. 127),

according to the authors probably (14–15). The first translocation and this latter therefore involved in common a chromosome D no doubt a 14 or a 15. This concordance suggested a cause and effect relation between the phenotypic anomalies and a juxta centromeric deletion of the large arm of a 14 or a 15 chromosome (Turpin and Lejeune, 1964a).

The second observation of G ~ D translocation was that of Moorhead, Mellman and Wenar (1961) in six children. The father and the fourth child possessed a normal karyotype. On the other hand, the mother and the first three children had a karyotype with 45 chromosomes and a translocation of a small onto a large acrocentric producing a picture quite comparable with that in Fig. 7.2.

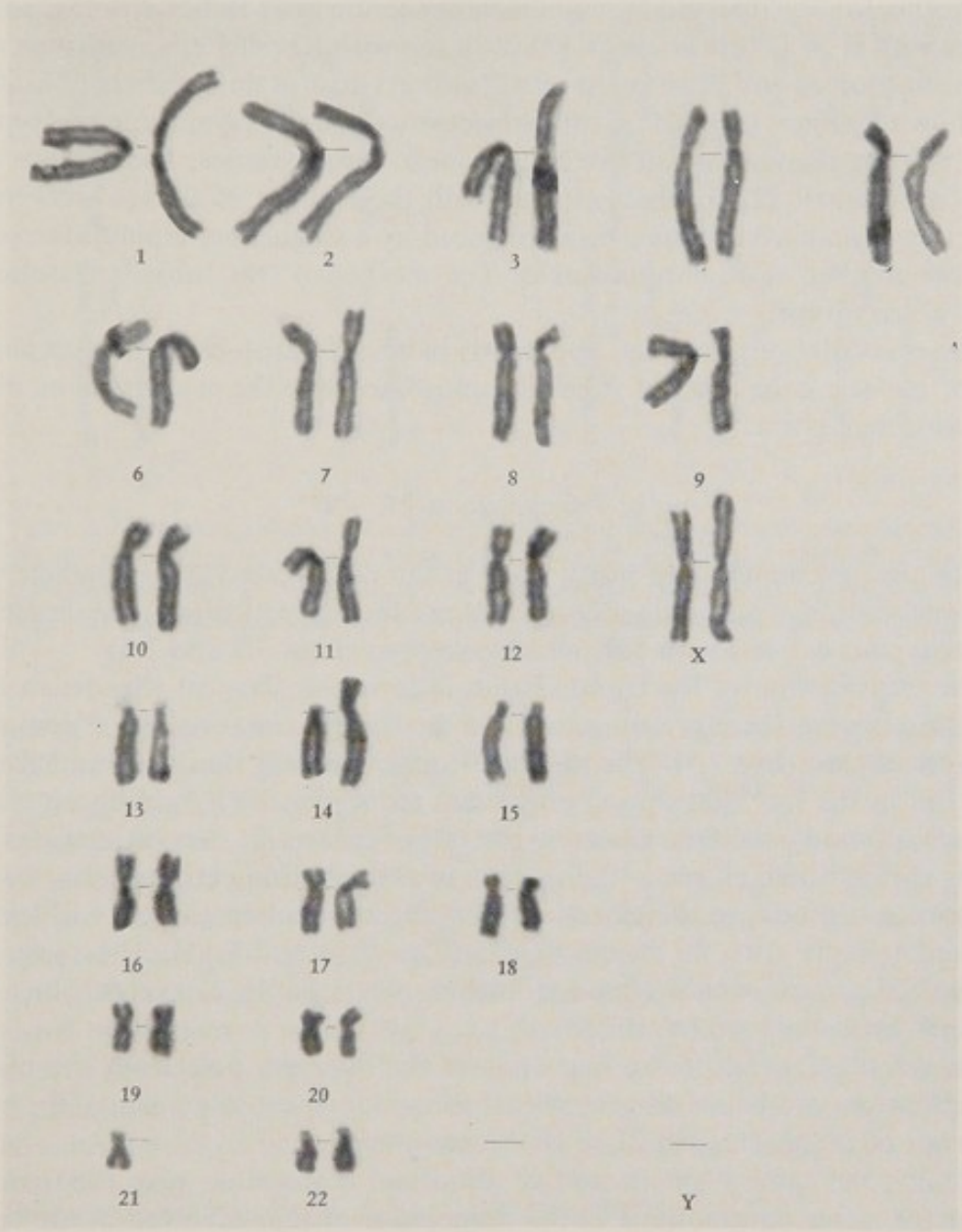


FIG. 7.8. Mother of a child with trisomy 21 (No. 1071). Note the 21 ~ D translocation.

Finally, the sixth child displayed a cytologically and clinically typical trisomy 21 with 47 chromosomes and apart from this free trisomy 21 showed no other karyotypic anomalies. In particular, this child did not possess the G ~ D translocation transmitted by his mother to four of her six children.

The identification of the small acrocentric as a 22 rests on morphological and genetic arguments, apparent presence of two 21 chromosomes with 45 chromosomes in the cells of the mother and the four children and absence of translocation in the patient with trisomy 21 with 47 chromosomes.

It should be noted that in this family the children with translocation showed serious mental retardation, particularly of speech, while the transmitting mother was at the lower limits of normal.

Another family observed in our laboratory concerned a father with 45 chromosomes with G ~ D translocation having a son with Klinefelter's syndrome with 46 chromosomes and bearing the translocation (Institut de Progénèse, No. 293). As shown by Figs. 7.5 and 7.6, this subject whose clinical syndrome was typical with positive chromatin had two X and one Y chromosomes. The absence of a small acrocentric (22) probably linked with the absence of a large acrocentric (13), these two chromosomes being replaced by a single one, explains the paradoxical number of 46 chromosomes. The mother of this subject possessed a normal karyotype.

These associations, between apparently balanced translocation and an anomaly of meiotic behaviour of other chromosomes raise the problem of an interchromosomal effect.

(b) Translocation 21 ~ D

This type encountered in many cases of familial trisomy 21 is characterized by translocation of a 21 onto a D without producing particular anomalies in the heterozygous carriers with balanced karyotypes (Figs. 7.7 and 7.8).

The transmission of the translocation follows the classical Mendelian laws and this feature therefore appears, as a first approximation, as a dominant cytological trait (Fig. 7.9). The particular importance of this structural aberration lies in the fact that during chromatic reduction the hybrid chromosome 21 ~ D is found in half the gametes. The fate of the free 21 may be irregular and if this chromosome moves to the same pole as the hybrid chromosome, a diplo 21 gamete is produced. Fertilization by a normal haploid gamete will lead to masked trisomy with 46 chromosomes (Figs. 7.10 and 7.11). The reciprocal "nullo" 21 gamete would after fertilization give a haplo 21 zygote which has still not been observed (cf. p. 133).

On the other hand, if the free 21 is at the opposite pole from the hybrid chromosome, two balanced gametes are formed, one entirely normal, the other a carrier of the rearranged 21 ~ D chromosome.

Finally, the same phenomenon of abnormal segregation may theoretically occur for a free chromosome D the homologue of that involved in the translocation leading to two types of gametes: diplo (D) giving trisomic D individuals and "nullo" D (giving haplo D individuals).

Of the six combinations only four are schematized in the appended figure. In fact, no case of abnormal segregation of a D chromosome has been reported in families with G ~ D translocation although it has been observed in cases of D ~ D translocation. This difference may be related to the larger mass of a D chromosome which would thus be more stable than the G during meiosis in heterozygotes with a G ~ D translocation.

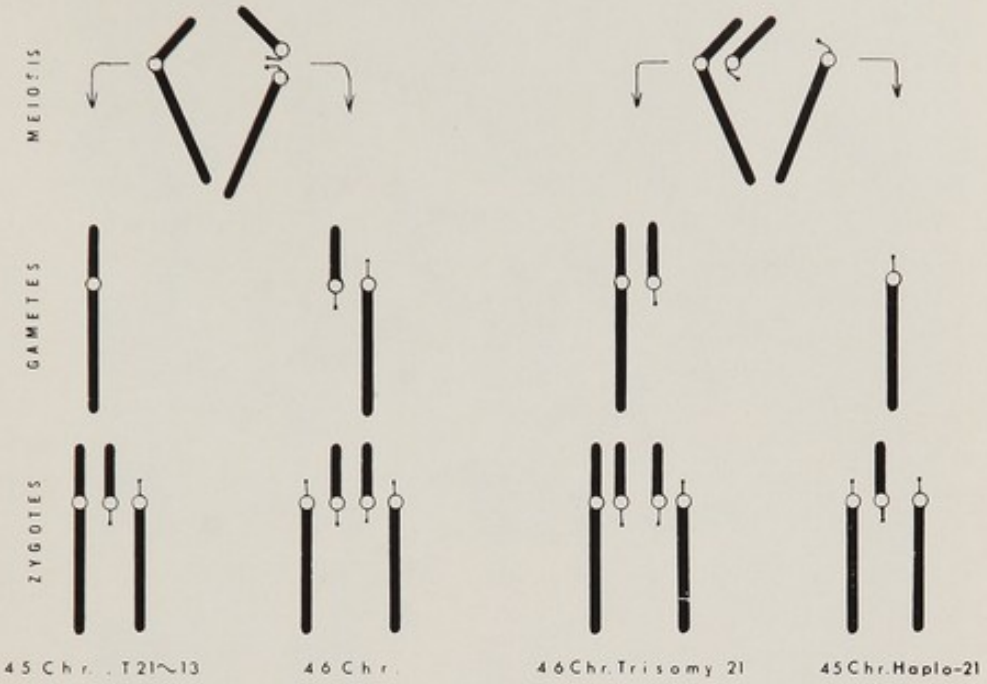


FIG. 7.9. Meiotic segregation of a 21 ~ D translocation. The progeny of a heterozygous subject are made up of three types of children: carriers (normal) of the translocation with 45 chromosomes; normal children with normal karyotype of 46 chromosomes; and trisomics 21 by translocation. Haplo 21 zygotes are probably not viable.

Finally, a simultaneous abnormal segregation of 21 and D would lead to nullo-21 and nullo-D gametes respectively and diplo-21 and diplo-D, that is, haplo-21 and haplo-D or triplo-21 and triplo-D zygotes.

Reported for the first time by Penrose *et al.* (1960), since the case of Polani *et al.* (1960) had parents with 46 normal chromosomes (Carter *et al.*, 1960), the familial transmission of the 21 ~ D translocation confirmed by the latter authors in another family is now well known.

Preferential transmission of the translocated chromosome by males has been suggested by Hamerton *et al.* (1961) and discussed by Sheppard (1961) but the evidence accumulated suggested to Hamerton and Steinberg (1962c) that this was a phenomenon peculiar to a given family as proposed by Shaw (1962a).

The cases described in the literature are summarized in Table 7.1.

(1) Progeny of a mother with a 21 ~ D translocation

Analysis of this table shows that the three types of children: normal with 46 chromosomes, normal with 45 chromosomes (by translocation) and tri-

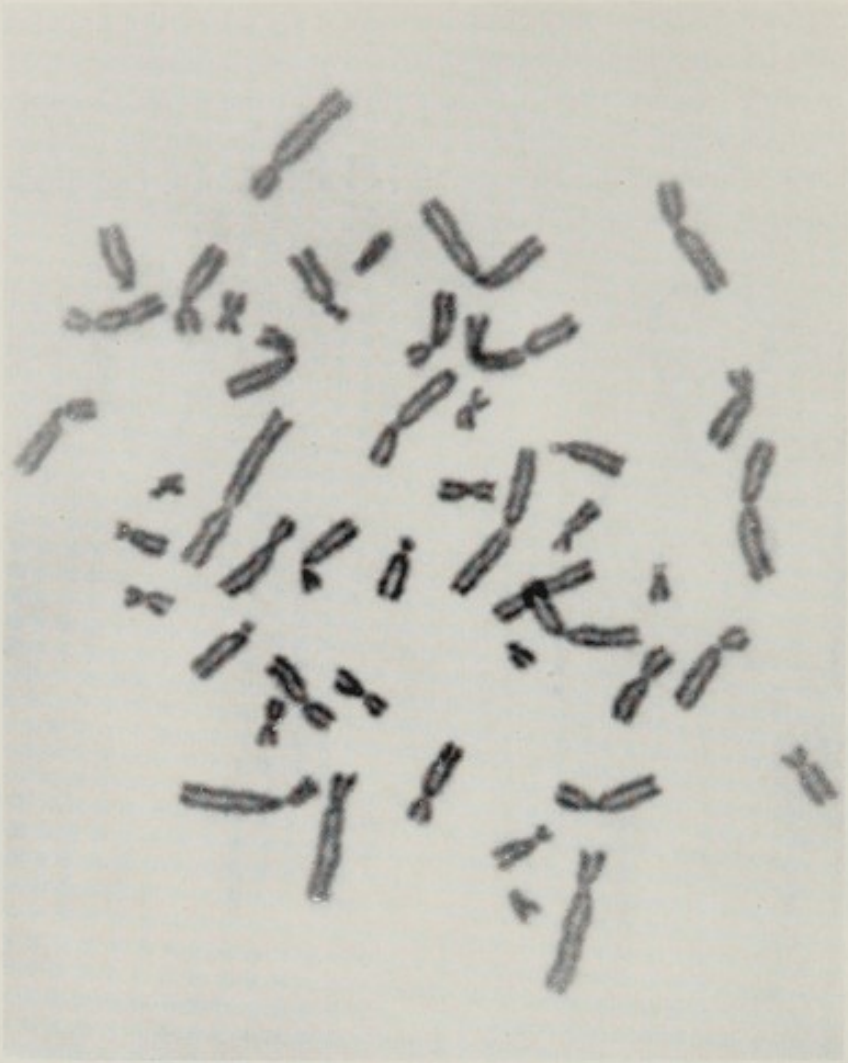


FIG. 7.10. Cell of a patient with trisomy 21 by 21 ~ D translocation. Son of previous subject (Figs. 7.6 and 7.7) (Institut de Progénèse, No. 1070).

TABLE 7.1

Progeny of subjects with a 21 ~ D translocation

Number of transmitting parents	Children						
	46 chr. N	45 chr. T	46 chr. tris. 21	Phenotype tris. 21	Normal phenotype	Miscarriages	Total number of children
Mothers: 39	30	30	33 (20)	13	14	16	120
Fathers: 16	15	22	2 (2)	1	10	?	50

Table based on cases reported by:

Atkins *et al.*, 1962c; Beçak *et al.*, 1963; Breg *et al.*, 1962b; Biesele *et al.*, 1962; Brandt *et al.*, 1963b; Buckton *et al.*, 1961; Carter *et al.*, 1960; Forssman and Lehmann, 1962; German *et al.*, 1962d; Hamerton *et al.*, 1961a, b; Hayashi, 1963; Hustinx, 1963d; Penrose *et al.*, 1960; Penrose and Delhanty, 1961d; Institut de Progénèse, 1964, No. 1070 and No. 1082; MacIntyre *et al.*, 1962; Sergovich *et al.*, 1962; Shaw, 1962b.

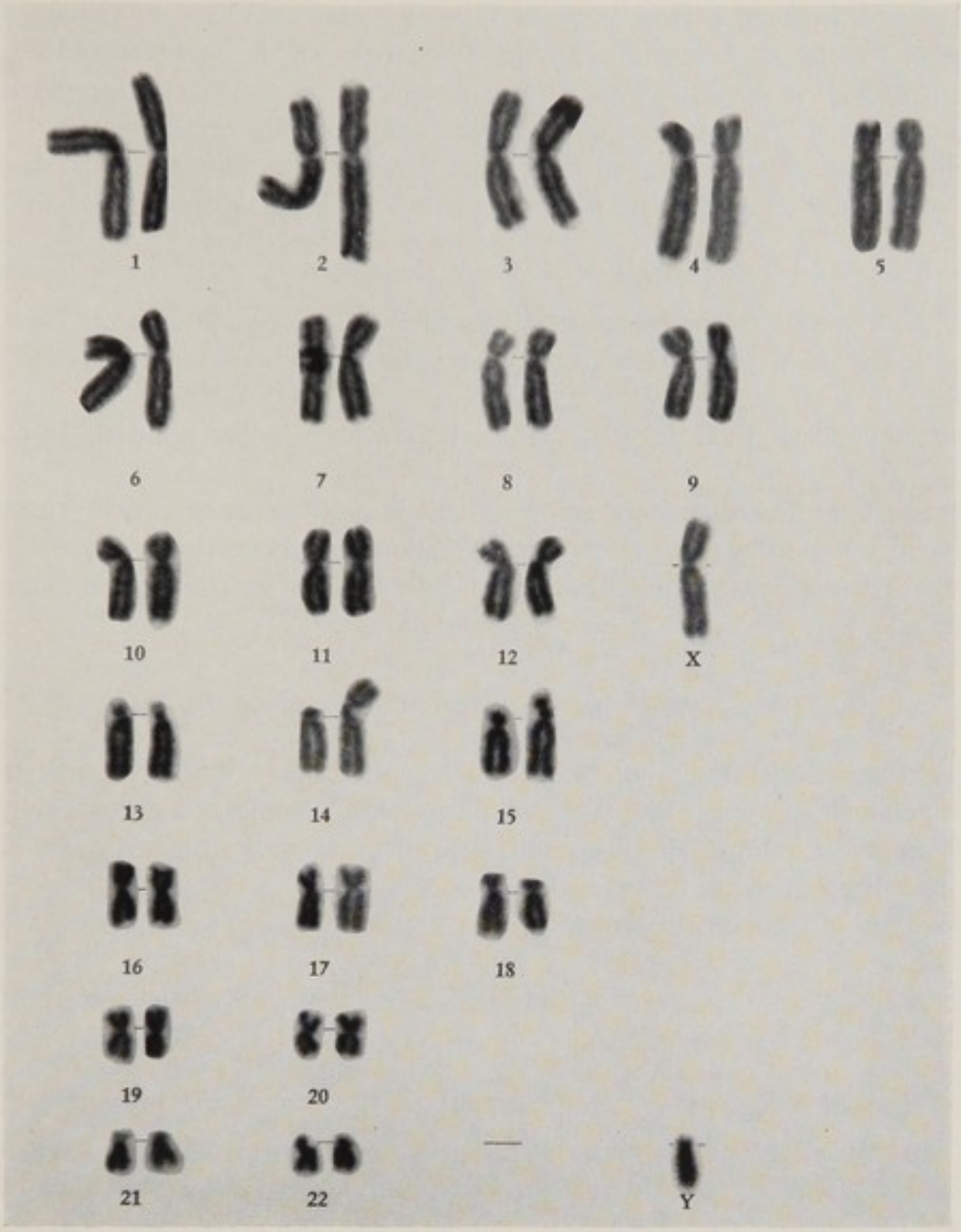


FIG. 7.11. Karyotype of a subject with trisomy 21 by 21 ~ D translocation. Son of previous subject (Figs. 7.6 and 7.7) (Institut de Progénèse, No. 1070).

somics 21 with 46 chromosomes (by translocation) appear with a more or less identical frequency.

The apparent predominance of trisomy 21 (33 + 13) must be corrected for the presence of 20 index patients and the 14 normal must be equally distributed into two categories, 46 N and 45 T.

If it is assumed that the haplo 21 zygotes were produced in a number comparable with that of trisomy 21 (the reported frequency of miscarriages cannot unfortunately be taken into consideration) it may be concluded that the chromosome 21 segregates abnormally in one in two.

In other words, in a heterozygous G ~ D female, the free 21 chromosome migrates by chance sometimes to the same pole as the translocated 21 and sometimes to the opposite pole and existence of the translocation appears to upset completely the regularity of the mechanism of segregation of 21.

From the progenesic point of view these findings show that the risk of appearance of trisomy 21 in descendants of a mother with translocation is 1 in 3.

(2) Progeny of a father with a G ~ D translocation

The distribution of the three categories of children is quite different here and only one case of trisomy 21, index patients excluded, has been reported among 48 sibs.

The transmission of the translocation appears to occur regularly in one in two.

This striking difference between males and females in the meiotic disturbance produced by translocation cannot be explained simply at the present time; it perhaps corresponds to a fundamental difference in meiosis in the male and the female.

(c) Translocation between two small acrocentrics (G ~ G)

The centromeric fusion of two small acrocentrics produces a new metacentric element the morphology of which strongly resembles a chromosome 19 or 20. In fact, the long arm of a small acrocentric is more or less the size of the arm of a 19 or 20 (Fig. 7.12).

This type of translocation raises the same problems of identification as type D ~ G.

Here, however, there exist only three possible G ~ G varieties, namely, 21 ~ 21, 21 ~ 22 and 22 ~ 22.

(1) Variety 21 ~ 21

When one of the acrocentrics affected by the rearrangement is a chromosome 21, transmission of the translocation may lead to the appearance of trisomies 21 with 46 chromosomes.

The first observation of this kind was reported by Fraccaro *et al.* (1960) in a child with a typical clinical syndrome of trisomy 21. The karyotype revealed 46 chromosomes including two chromosomes 22 and only one 21, the figure 46 being restored by the presence of a small metacentric quite similar to a 19–20 interpreted as a fusion of two 21 or one 21 and one 22 chromosomes. Another case published by Hamerton *et al.* (1961a) presented a comparable karyotype. These two observations can be related since in both cases the father of the trisomy 21 patients showed a mosaic of cells with 46 normal chromosomes (majority) and others with 47 chromosomes the supernumary being, in both cases, a small metacentric of type 19–20. In these two observations it seems plausible to consider that the paternal mosaic extended to germinal tissue giving transmission of an extra metacentric. It should be noted that the trisomy 21 patient reported by Hamerton *et al.* also displayed a mosaic with the presence

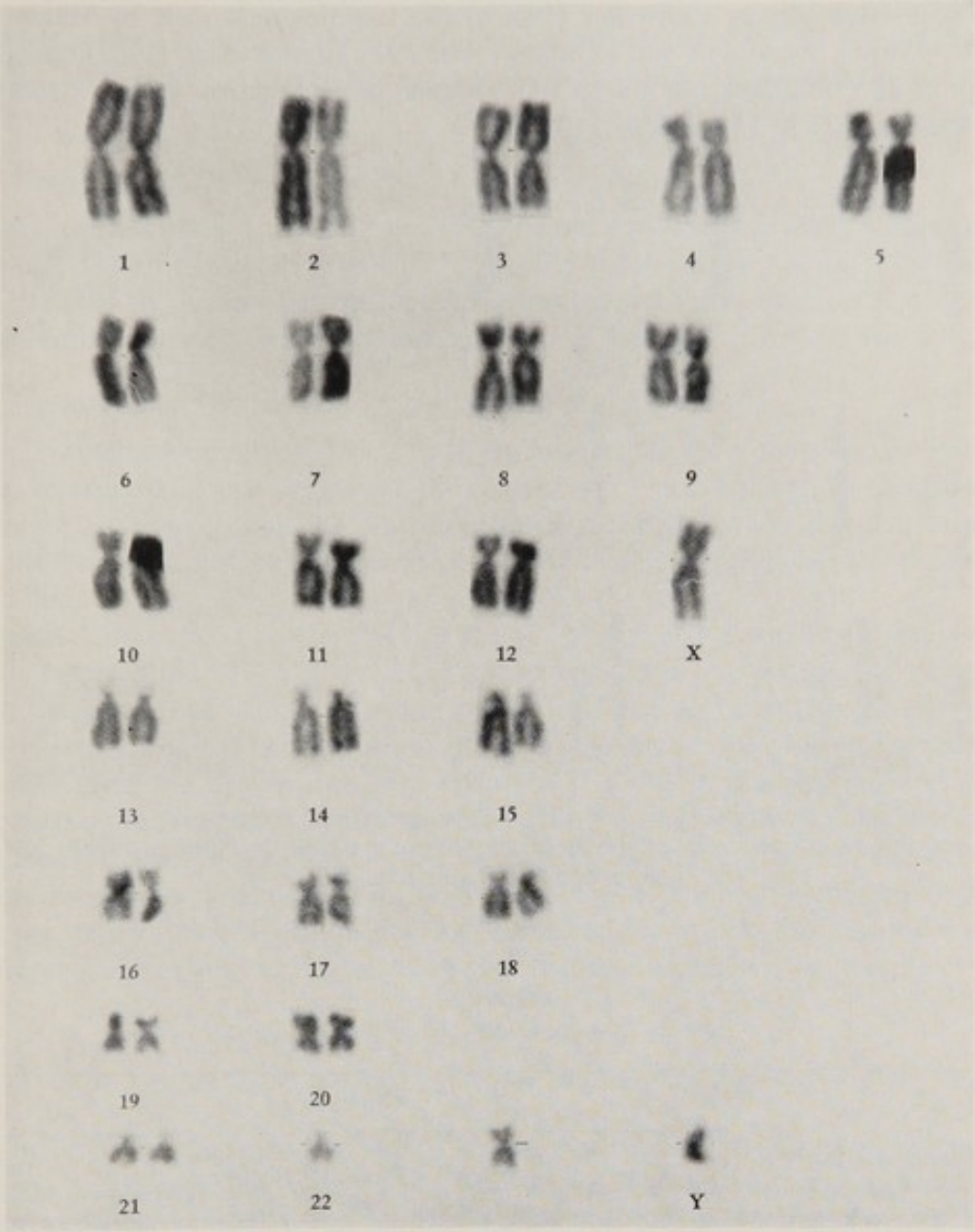


FIG. 7.12. Karyotype of a trisomic 21 with G ~ G translocation. (Photograph by courtesy of Dr. Jean de Grouchy.)

of rare cells with 47 chromosomes identical with those of the paternal clone. Such a phenomenon suggests instability of the karyotype during the early embryonic period.

The importance of the identification of the two elements involved in the translocation derives from the fact that an individual heterozygous for a 21 ~ 21 translocation can only produce diplo 21 (carriers of the translocation) or nullo 21 gametes. In the last analysis, as shown by the diagram (Fig. 7.13) the progeny of such a subject can only consist of children with trisomy 21, the haplo 21 zygotes probably being non-viable (cf. p. 133).

As pointed out by Zellweger (1962b) the families described by Hamerton *et al.* (1961a), Forssman and Lehman (1962), Mukherjee *et al.* (1962) and Dallaire *et al.* (1962) correspond to this absence of segregation giving a total of 13 trisomies 21 in 13 children.

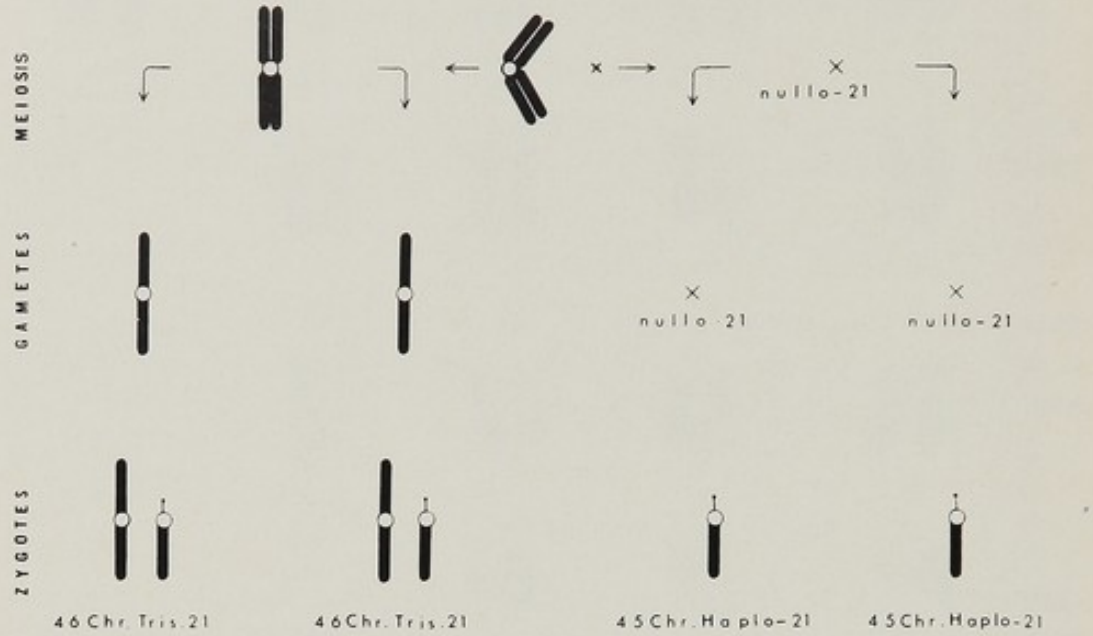


FIG. 7.13. Meiotic segregation of a 21 ~ 21 translocation. The progeny of a carrier of this translocation is composed exclusively of trisomic 21 children, haplo 21 zygotes probably being non-viable.

TABLE 7.2

Progeny of subjects with a G ~ G translocation

Transmitting parent		Children						
		46 chr. N	45 chr. T	46 chr. tris. 21 by transloc	Phenotype tris. 21	Normal phenotype	Miscarriages	Total number of children
Variety 21 ~ 21	Mothers: 2	0	0	3	2	0		5
	Fathers: 2	0	0	4 (1)	4	0	6	8
Variety 21 ~ 22	Mothers: 3	1	4	2 (2)	0	0	3	7
	Fathers: 5	8	7	1 (1)	0	4	1	20
Mosaicism G~G in one of the parents	Mothers: 2	0	0	2 (2)	1	4		7

Based on cases reported by: Dallaire *et al.*, 1962; Fraccaro *et al.*, 1960h; Forssman and Lehmann, 1962; Hamerton *et al.*, 1961a; Mukherjee *et al.*, 1962; Pfeiffer, 1963a; and Shaw, 1962b.

The impossibility of cytological identification of the 21 ~ 21 variety is particularly serious since with the risk of appearance of trisomy 21 at 100 per cent, one can appreciate the eugenic importance of preventive detection of this variety of G ~ G translocation.

(2) Variety 21 ~ 22

On the other hand, variety 21 ~ 22 may be transmitted in a family with a very low risk of appearance of trisomy 21 (families mentioned by Shaw [1962] and Pfeiffer [1963]). It may be considered that the meiotic disturbance is more or less comparable with that produced by a 21 ~ D translocation (cf. Fig. 7.8).

(3) Variety 22 ~ 22

Individualization of the 22 ~ 22 variety has not yet been achieved. As in the case of variety 21 ~ 21, progeny of the heterozygous carriers must be exclusively trisomic for chromosome 22. The characteristics of this syndrome, if it exists, are little known (cf. Chapter 6) and no case of familial segregation has yet been recorded.

Table 7.2 summarizes the findings in the literature to date. The distinction made between the 21 ~ 21 and 21 ~ 22 varieties based on progeny of parents with translocations is only probable and cannot be categorically asserted.

The importance of translocations in the progenesis advice which it is possible to offer after the appearance of a trisomic 21 child in practice requires the systematic examination of patients with trisomy 21 and their parents in order to detect families in which there is a very great risk of recurrence of the defect either because of a translocation or a mosaic. The latter problem cannot yet be completely treated because of the present paucity of information but it too represents a very important factor in establishing a eugenic prognosis.

(d) Translocation between two large acrocentrics (type D ~ D)

The first translocation between two large acrocentrics was observed in a patient with typical Klinefelter's syndrome XXY. This patient, despite an extra X chromosome, presented a paradoxical number of 46 chromosomes owing to the absence of two large acrocentrics and the presence of an extra large mediocentric quite similar to chromosome 3 but perfectly symmetrical in relation to the centromere. This chromosome was interpreted for morphological and genetic reasons as a case of 14 ~ 15 translocation (Lejeune, Turpin and Decourt, 1960c) (Figs. 7.14 and 7.15).

A very fine observation of familial transmission of a D ~ D translocation without an important phenotypic effect was reported by Walker and Harris (1962a, b, c). In this family the presence of nine carriers of the translocation (proposita excluded) and of eight subjects with 46 chromosomes demonstrates equality of transmission of the rearranged chromosome and its two free homologues. This family also indicates that the translocated chromosome must be a hybrid (13 ~ 14, 13 ~ 15 or 14 ~ 15) and cannot be an isochromosome (13 ~ 13,

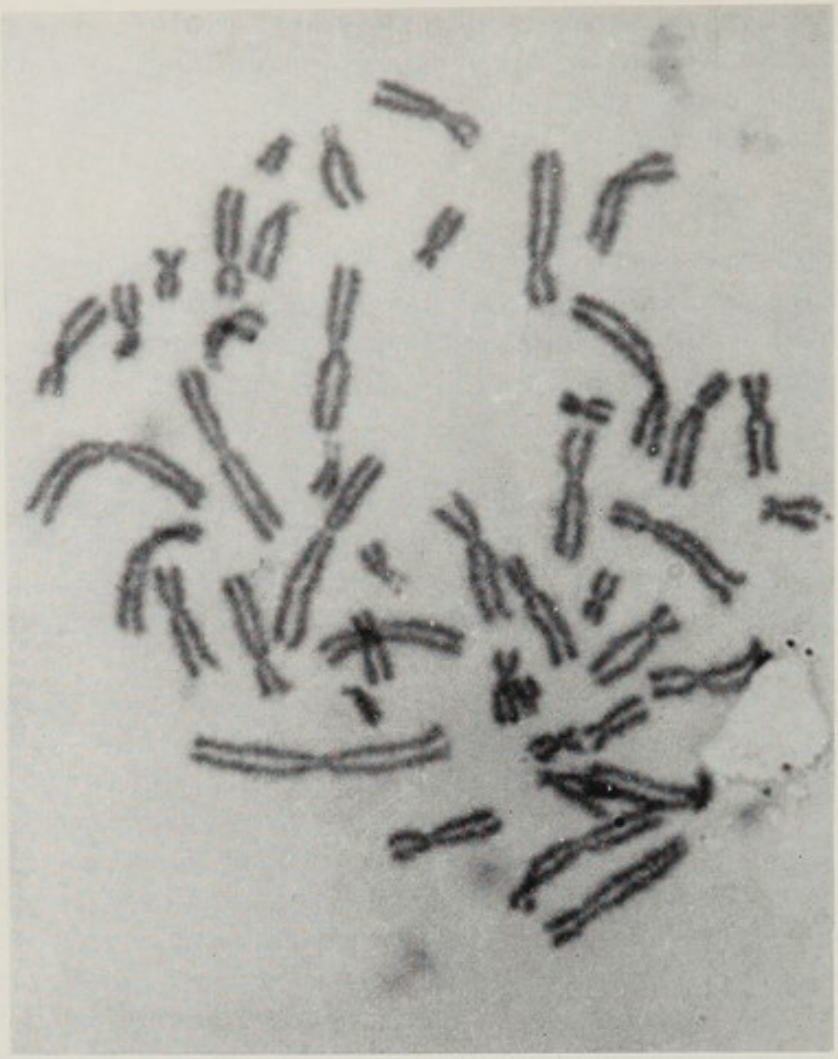


FIG. 7.14. Cell of a subject with D ~ D translocation plus XXY.

TABLE 7.3

Progeny of a parent with a D ~ D translocation

Transmitting parent	Children						
	46 chr. N	45 chr. T	Tris. D with 46 chr.	Normal phenotype	Anomaly differing from a tris. D	Miscarriages	Total number of children
Mothers: 8	12	8	1	2	1	4	24
Fathers: 5	1	6	1	1			9
Transmitting parent not examined: 2	4	6	0	6			16
	17	20	2	9	1	4	49

Based on cases reported by: Bowen *et al.*, 1963; Dill and Miller, 1963; Grouchy *et al.*, 1963k; Hamerton *et al.*, 1963a; Oikawa *et al.*, 1962; Walker and Harris, 1962b and c.

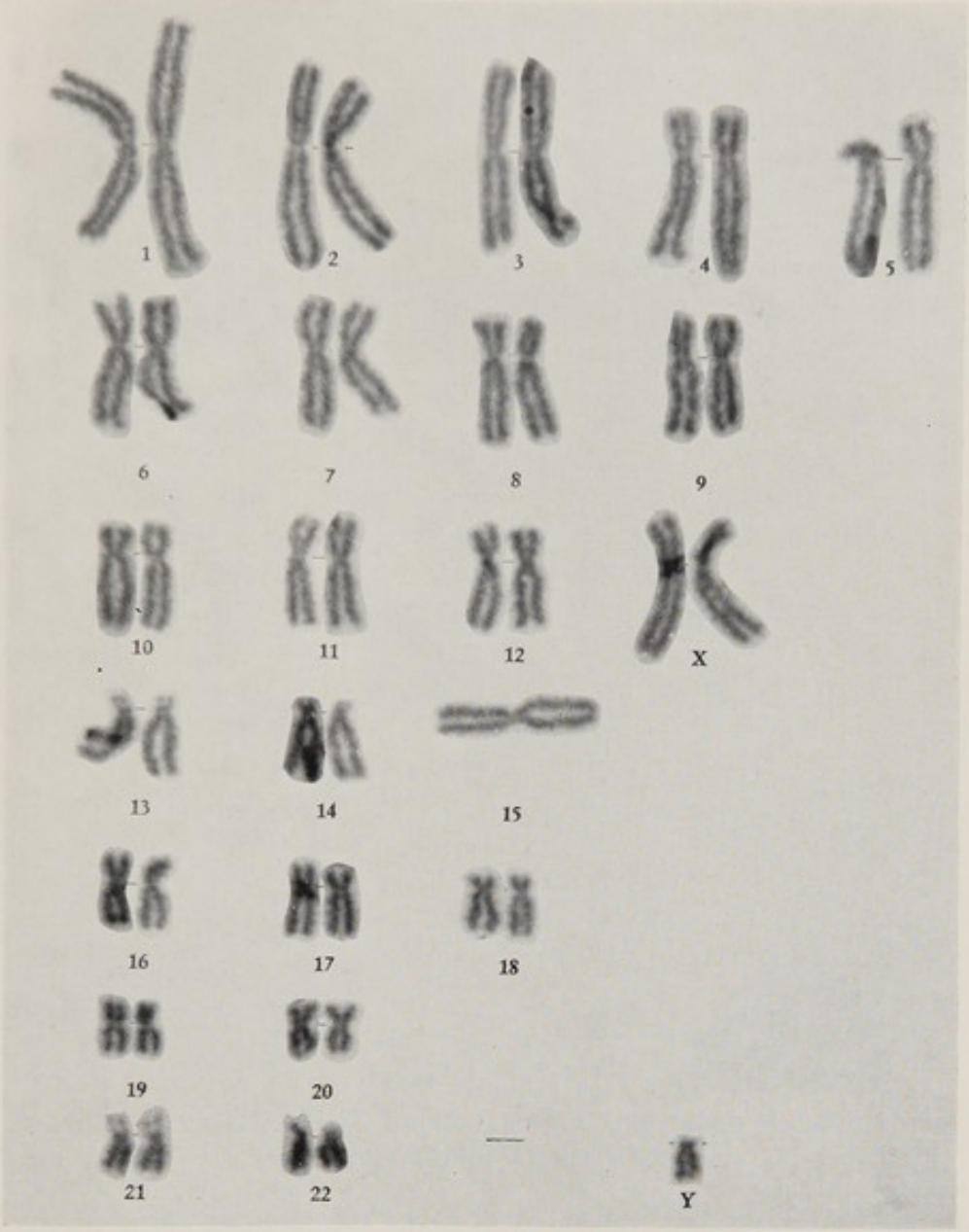


FIG. 7.15. Karyotype of XXY subject with D ~ D translocation.

14 ~ 14 or 15 ~ 15). These three combinations would lead inevitably, as shown by the diagram (Fig. 7.13) for a 21 ~ 21 translocation, to a trisomy for one of the large acrocentrics coupled with monosomy for another, an imbalance incompatible with an apparently normal phenotype.

Other cases of familial segregation of a D ~ D translocation have been reported by Oikawa *et al.* (1962), Grouchy *et al.* (1963k) (already mentioned in § (a)), Hamerton *et al.* (1963a), Dill and Miller (1963) and Bowen *et al.* (1963) and all the progeny are listed in Table 7.3.

If we exclude the two subjects with trisomy 13 by translocation (cf. Chapter 6) the number of children with D ~ D translocation is comparable with that of those with a normal karyotype.

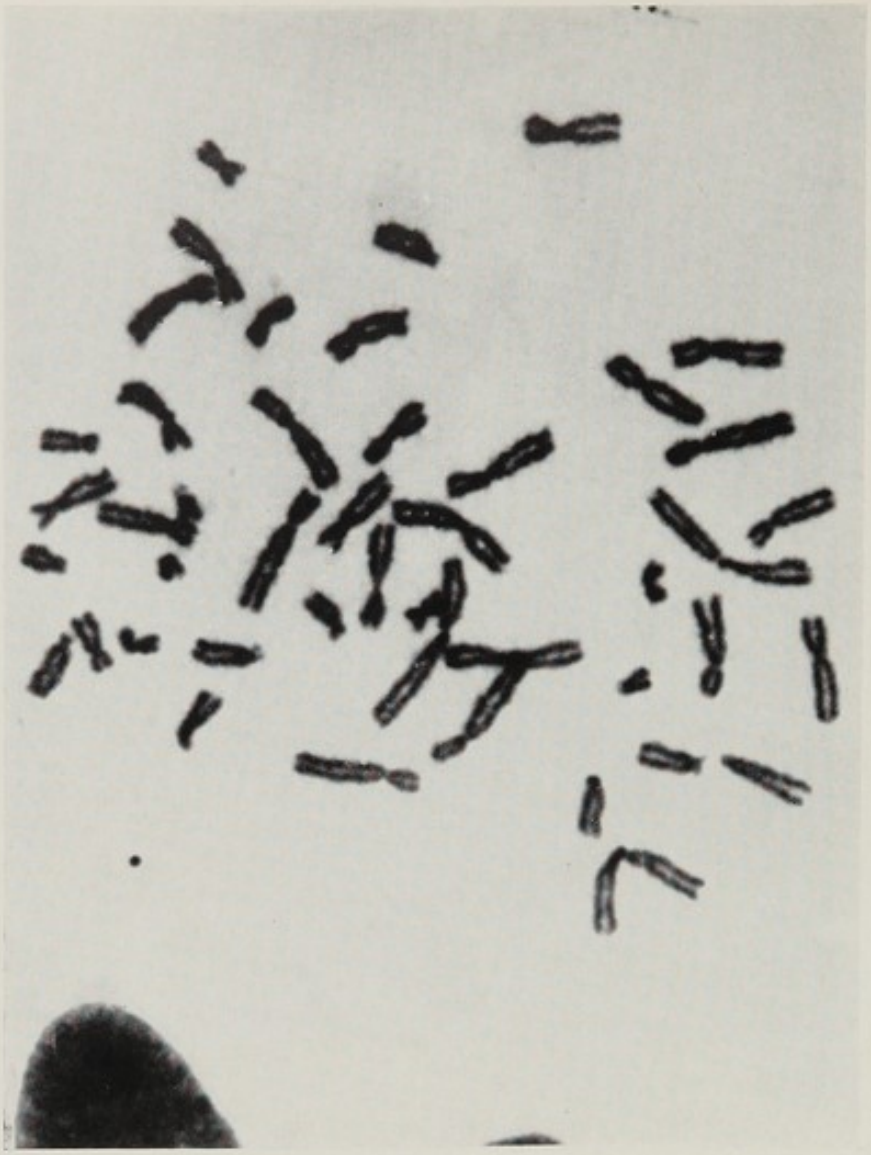


FIG. 7.16. Cell of a subject with Turner's syndrome, haplo X and with 2 ~ G translocation (Institut de Progénèse, No. 420).

Although a preferential transmission of the translocated chromosome was considered by Bowen *et al.* (1963) the findings as a whole are in quite good agreement with a normal 1:1 segregation.

This type of D ~ D translocation has also been sporadically observed in normal individuals examined systematically (Makino *et al.*, 1961 b; Cooper and Hirschhorn, 1961 b) or those presenting an associated anomaly: psychomotor retardation (Ferguson and Pitt, 1962); mental retardation and cryptorchidism (Bühler *et al.*, 1963); case of mosaicism with normal cells and cells with 45 chromosomes by D ~ D translocation observed in a mentally defective child (Turpin and Lejeune, 1964a).

The possible causal relation between the translocation and the phenotype is very difficult to define in these cases. In this connection it should be noted that the patient whose condition led to the examination of the family mentioned by Walker and Harris showed primary amenorrhea of undefined origin.

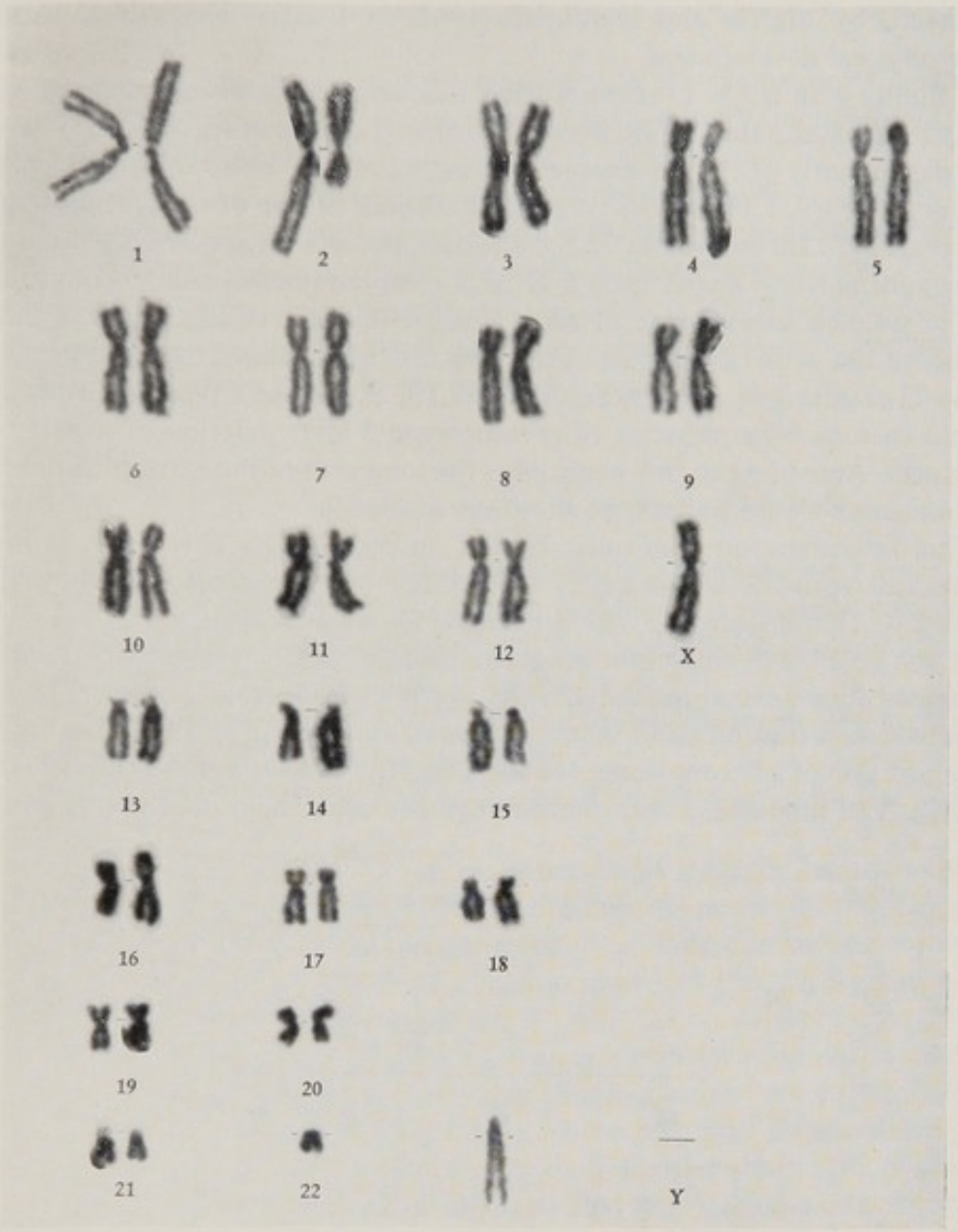


FIG. 7.17. Karyotype of a subject with haplo X Turner's syndrome and 2 ~ G translocation (Institut de Progénèse, No. 420).

These facts taken as a whole suggest that in the general phenotypically healthy population the frequency of the D ~ D translocation may not be altogether negligible although we cannot yet say whether this is a balanced polymorphism because of a selective advantage conferred by translocation or a simple mutational burden maintained simply by the frequency of chromosome mutations.

(e) Translocation between an acrocentric and a chromosome of another type

A karyotype with 45 chromosomes through absence of a D chromosome and presence of a chromosome 2 the long arms of which were twice normal size was

observed, by Mercer and Darakjian (1962) in a backward subject showing poor physical development.

A family with a 2 ~ G translocation was detected by examination of a subject with typical haplo X Turner's syndrome (Lejeune *et al.*, 1963a).

Independently of the sex anomaly (presence of only one X chromosome) the karyotype (Figs. 7.16 and 7.17) revealed absence of one of the chromosomes 2 and one of the chromosomes 22 and the presence of a large acrocentric chromosome substantially longer than a D plus a supernumary in the 6–12 group.

The simplest explanation of such a rearrangement consists of imagining a translocation of the distal part of the long arm of chromosome 2 onto chromosome 22 resulting in an extra acrocentric. The extra medium-sized chromosome would then be what remains of chromosome 2 after deletion of a part of its long arm. According to this hypothesis the long arm of the extra medium-sized chromosome would, in fact, be the short arm of 2.

This interpretation confirmed by the absence of sex chromatin is in full agreement with the karyotype of the mother of the patient who showed the same 2 ~ 22 translocation with a normal XX sex complement.

It was possible to study four generations of the family revealing in 11 subjects examined four normal individuals and seven with the translocation (Fig. 7.18).

A balanced translocation between a small acrocentric and the distal part of the short arm of a chromosome 4–5 was observed by Gustavson *et al.* (1964) in a female. The modified 5 was morphologically identical with the chromosome

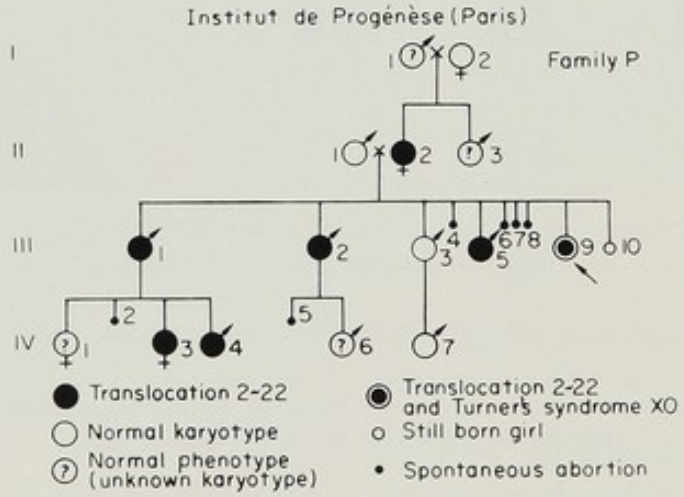


FIG. 7.18. Family No. 420 (Institut de Progénèse) showing the segregation of the 2 ~ G translocation (After Lejeune *et al.*, 1963).

previously described in the syndrome of "deletion of the short arm of 5" (cf. Chapter 6) and the small acrocentric which received the fragment became a small metacentric very similar to a chromosome 19–20. There were only three small normal acrocentrics.

In a boy born to this woman, chromosomes 4–5 were normal and the small metacentric observed in the mother was additional to the four small acro-

centrics (including the Y). This child would therefore be trisomic for the translocated fragment of chromosome 5.

It may be that this situation is the reciprocal of the syndrome of deletion of chromosome 5 in the light of the conditions known in *Drosophila* as haplo IV and triplo IV (cf. Chapter 6).

The child who died at the age of 3·5 months had a small jaw, a slight hypertelorism and low-set and mis-shapen ears. He showed neither epicanthus, nor a transverse palmar crease but had a membranous imperforate anus. There was only a single umbilical artery present, with, in addition, bilateral iridocolobomata with blepharophimosis. There was no microcephalia but, on the contrary, the cranial perimeter was 59 cm at the age of 3·5 months.

(f) Translocation between metacentric chromosomes

This last type is much more difficult to identify morphologically since a reciprocal translocation between the 6 ~ 12 elements, for example, can only be detected if the rearrangement leads to a very marked difference in size or to obvious change in the centromeric index.

It is probable that a considerable number of rearrangements between metacentric chromosomes are totally unnoticed.

Edwards *et al.* (1962) observed two cases of transmission of a translocation between metacentrics.

The first family consisted of two malformed and mentally retarded children whose karyotypes revealed an excess of chromosome material on the long arm of chromosome 4. The father also showed a too long chromosome 4 and, in addition, one of his medium-sized chromosomes had lost its short arm, thus giving an acrocentric resembling a D.

The explanation proposed by the authors of a translocation of the short arm of a medium-sized chromosome (9) onto the long arm of a 4 accounts for the normal phenotype of the father, carrier of the balanced translocation 9 ~ 4 while the children would, in fact, be trisomic for the short arm of chromosome 9. It should be noted that this trisomy by translocation was accompanied by dermatoglyphic irregularities; fusion of the triradii b and c, presence of a hypothenar formation, P 14 and bilateral palmar creases. These signs were present on both hands in the two children.

In the second family a reciprocal translocation between a chromosome 1 and a chromosome 6 was transmitted by a father to his daughter who, receiving the translocation and the normal chromosome 1, was thereby trisomic for the translocated fragment. The mentally retarded child showed a forehead with a very developed median eminence with hypoplasia of the anterior temporal region.

The morphological anomaly of one chromosome 1 in a boy with severe hypospadias has been reported by Cooper and Hernits (1963). The presence of a too long arm of a chromosome 1 was found in his mother and one of his sisters, both phenotypically normal. A twin sister of the index patient showed a normal karyotype and phenotype.

Possible relations between the excess length of chromosome 1 and the intersex state of the index patient were not claimed by the authors. However, the hypothesis of translocation of a portion of an X chromosome onto a chromosome 1 is conceivable.

(g) Other structural rearrangements

The existence of other types of rearrangement, apart from translocations and deletions mentioned in Chapter 6, has been postulated in several observations.

Pericentric inversion

A pericentric inversion of 21 is assumed by Grey *et al.* (1962) in the mother of a trisomic 21 through 21 ~ G translocation.

Probable pericentric inversion of one of the D chromosomes giving a chromosome with distal centromere with well-individualized satellited short arms is reported in a father and daughter, both phenotypically normal.

No case of simple inversion is yet known, this absence being probably the reflection of the technical impossibility of detecting an anomaly which quite probably exists in our species.

Ring chromosomes

The rearrangement leading to ring chromosomes stable during mitotic division has been observed several times. The letter r following the number of the chromosomes involved may be proposed to describe this type of aberration.

Several cases concern the X chromosome. The first case to be reported was described by Lindsten and Tillinger (1962c) and showed a certain instability being present only in the blood and not the skin (cells with 45 chromosomes, haplo X) in a patient with gonadal dysgenesis. Another case of mosaicism for a ring X chromosome was reported by Lüers *et al.* (1963b) and the case of Smith–White *et al.* (1963) may also be related to a rearrangement of an X chromosome.

Two cases involving chromosome 18 have been reported. Lucas *et al.* (1963) described a mentally retarded and slightly microcephalic child with cleft palate and dislocation of the hip without other malformations. One of the chromosomes 18 was replaced by a very small ring. The patient described by Genest *et al.* (1963b) showed a ring 18, quite similar to the preceding one and an excess of chromosome material on the short arms of a D chromosome. This child with hypoplasia of the middle ear, deafness and imperforate external auditory meatus, had an epicanthus, hypertelorism and club feet.

These two cases may be compared with the rearrangements mentioned in trisomy 18 and deletion of the short arm of 18 (cf. Chapter 5).

The observation of a case of deletion of the long arm of 18 (Grouchy *et al.*, 1964d) and a new case of a ring 18 (Grouchy *et al.*, 1961b) allowed this author to consider the factorial analysis of this chromosome.

Finally, a small ring acrocentric was recently observed (Lejeune *et al.*, 1964e) in a boy with a mosaic of haplo-G cells with 45 chromosomes and cells with 46 chromosomes bearing a small ring acrocentric. The anomalies seem to be the countertype of the stigmata in trisomy 21. This observation suggests that monosomy 21 held to be non-viable from study of the familial segregation of translocations involving a 21 chromosome (pp. 118 and 123) may be observed in the form of a constitutional mosaic providing a new example of a reciprocal syndrome (p. 107).

CHAPTER 8

Leukaemias and Cancers

As the preceding chapters have shown, the early appearance of a chromosome aberration produces a change in the hereditary patrimony manifest in a constitutional disorder of the individual.

A later incident, during adult life for example, culminates in the formation of a "clone" all the cells of which carry the aberration which had appeared in their common ancestor. This clone thus has a different heritage from that of the individual who bears it. The variant cells are no longer distributed by chance within the normal cells as in constitutional mosaics but are localized topographically.

It is tempting to compare this notion of the mutant clone with the general process of malignancy and the present chapter is aimed at defining some current findings allowing us to draw or reject this parallel.

More than in any other field of cytogenetics prudence must be exercized in making statements and, above all, generalization. Only a few diseases have been sufficiently studied so far and the bulk of malignant conditions (solid tumours, in particular) still eludes systematic observation. This is to say in advance that the notions it is possible to draw from the current findings are only provisional and represent much more a plan of action for the pursuit of research than an attempt at elaborating a new theory.

The search for a genetic substratum for neoplastic transformation is by no means a new idea since as far back as 1914, Boveri, impressed by the multipolar mitoses discovered by von Hansemann (1890) in cancerous tissues proposed his famous hypothesis which can be summarized as follows: in tumour cells there is a specific chromosome change responsible for the new qualities of the malignant tissue.

Supplemented by the hypothesis of Winge (1930) who postulated cell selection as a function of the anomalies produced, the clonal theory of chromosome aberrations in neoplasms has since been the subject of lively discussion. Some workers consider with Bayreuther (1960) that chromosome anomalies are a late consequence of the malignant process. Others such as Makino (1957) and Hsu (1961) have made a very precise study of the concept of stem line showing a specific karyotype from which the other karyotypes may simultaneously derive. A great deal of work has been done in describing the karyotypes found in certain tumours (Levan, 1956a, b). Most of the observations unfortunately concern experimental and transmissible tumours (Ford *et al.*, 1958a, c) and not human spontaneous cancer.

If this chapter sought to outline all the facts available it would be practically impossible to do so within the limits of the present book.

Therefore, the observations on viruses and also experimental neoplasms in animals will be deliberately excluded and only reasonably established cytogenetic evidence in man presented.

A. ASSOCIATION BETWEEN THE NEOPLASTIC PROCESS AND CONSTITUTIONAL ANOMALIES

(a) Association in the same individual

Even before the chromosome determinism of trisomy 21 was discovered the existence of an association between the clinical condition and acute leukaemia was observed and demonstrated statistically (cf. Chapter 4).

That patients with trisomy 21 have a twenty times greater chance than normal individuals of developing acute leukaemia cannot be considered a mere coincidence but the explanation of this sensitivity cannot yet be formally given.

In view of the observations of Schade *et al.* (1962) association between acute leukaemia and the other trisomies, in particular, trisomy 13, is also possible.

The other chromosome anomalies, especially the sex aneuploidies, do not straight away reveal a marked association with malignant conditions. Nevertheless, some cases have been reported: (Mamunes *et al.*, 1961 [XXY subject with acute myeloblastic leukaemia]; Lewis *et al.*, 1963e [acute myeloblastic leukaemia in an X0/XXX female]; Bousser and Tanzer, 1963 [acute lymphoblastic leukaemia in a subject probably affected by Klinefelter's syndrome]; and Toughe *et al.*, 1962a [acute leukaemia in a mosaic XY/XXY male]).

Because of the relative frequency of sex aneuploidies it is too early to claim that subjects affected by them display leukaemia more often than would be accounted for by mere chance.

A partial deletion of one of the chromosomes 13–15 was observed by Lele *et al.* (1963b) in a girl with retinoblastoma. This latter observation suggests the long-known association between tumour processes and certain genetically transmitted disorders. Skin cancer in subjects with xeroderma pigmentosum, sarcomatous transformations in Recklinghausen's disease, chondrosarcomas of multiple osteochondromas, cancer developing on a colic polyps and gliomas in Bourneville's tuberous sclerosis are very well known examples.

None of these conditions seems to be related to a detectable chromosome anomaly and the observation of Lele (1963b) is the only one permitting detection of a chromosome aberration in retinoblastoma, a standard example of genetically controlled neoplasm.

All these associations between change in the hereditary patrimony and predisposition to tumours can hardly be explained at the present moment but they indicate that there exists a probably fundamental relation between the genetic material of the cells and neoplastic processes.

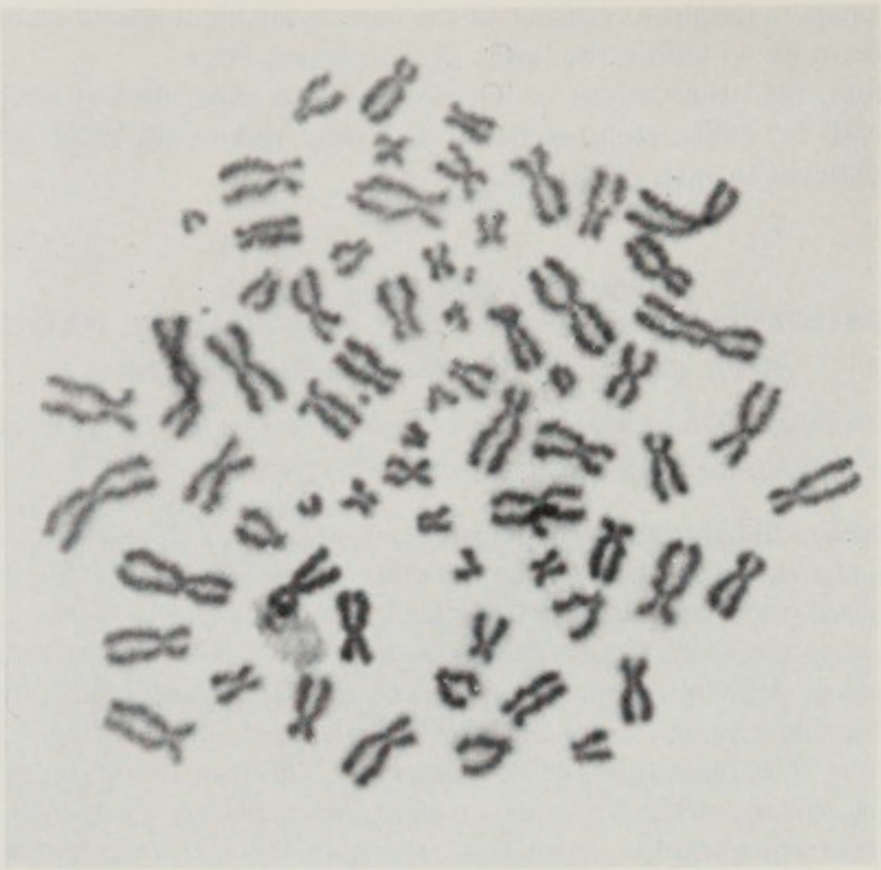


FIG. 8.1. Cell from a pleural neoplastic effusion (Institut de Progénèse, No. 531).

(b) Familial associations

Several families show a remarkable accumulation of leukaemias in the near relatives of a subject with aneuploidy. O. J. Miller *et al.* (1961 b) described a subject with chronic lymphoid leukaemia having an XXXXY son and also an aunt and cousin, both with trisomy 21. Baikie *et al.* (1961 e) report on a sibship of four children from normal parents including one case of acute lymphoblastic leukaemia, one of acute myeloblastic leukaemia and one XY/XXY mosaic; the other had died at an early age from acute bronchopneumonia. Buckton *et al.* (1961) mention a sibship of four children whose mother had a 21 ~ D translocation. One is trisomic 21 with 46 chromosomes (by translocation inherited from its mother); two died from bronchopneumonia and another from acute leukaemia. Hungerford and Nowell (1961) mention a leukaemia with blast cells in the brother of a trisomic 21. Thomson *et al.* (1963) report the case of a woman with acute lymphoblastic leukaemia, with a brother and a child with trisomy 21. Finally, Gunz *et al.* (1962) report the existence of an abnormal small acrocentric in two families presenting several cases of chronic lymphoid leukaemia.

The statistics of R. W. Miller (1963) likewise show that in sibships tainted with leukaemia, trisomy 21 is much more often encountered in the sibs than expected by chance and that the frequency of the malignant tumours in the sibs is also much higher than in healthy families.

All these familial concentrations raise the problem of the existence of a cause

FIG. 8.2. Karyotype of a pleural neoplastic effusion (Institut de Progénèse, No. 531).

common to these two sets of facts: constitutional chromosome anomalies, on the one hand, and leukaemias, on the other. The possibility of a causal relation, in particular through an interchromosomal effect, has been discussed (Lejeune, 1962c).

B. KARYOTYPIC STUDIES OF CERTAIN SOLID TUMOURS

Although it forms the subject of very many publications, the cytogenetic study of human tumours has still a very long way to go. Earlier work on knowledge of the normal human karyotype could not attain sufficient precision despite the

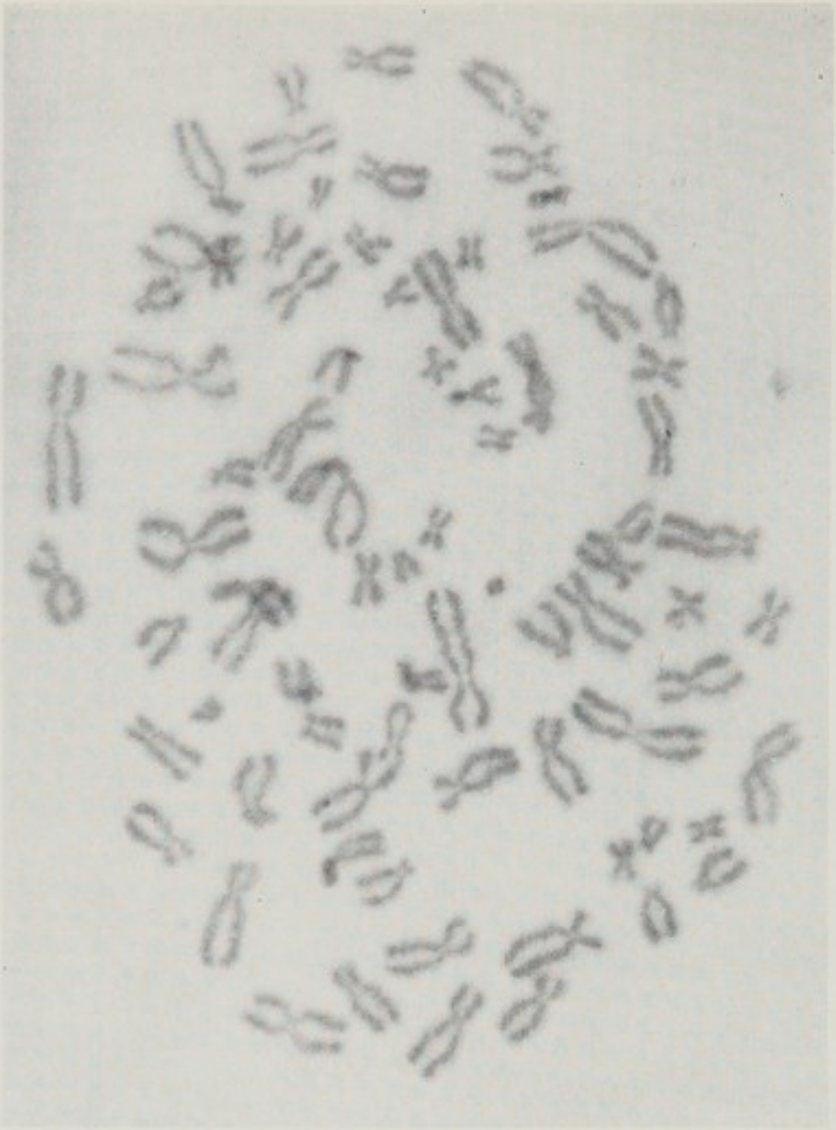


FIG. 8.3. Cell from a brain tumour (Institut de Progénèse, No. 800a).

quality of the observations. For example, the investigations of Fritz-Niggli (1954, 1955, 1956), Levan (1956a), Ising and Levan (1957) and Yoshida and Tabata (1957), to mention only a few, revealed a more or less marked aneuploidy or structural anomalies (Levan, 1956b) but this study could make little further progress.

Knowledge of the normal human karyotype gained since 1956 and of the main constitutional anomalies since 1959 has paved the way towards cytogenetic study of tumours.

However, despite the efforts made it is remarkable to find how few spontaneous human tumours have been studied completely. Certain observations of Ishihara (1959), Goodlin (1962), Spriggs *et al.* (1962b) and Lubs and Clark (1963) relate to examination of the solid tumour itself but so far only neoplastic effusions (ascites, pleurisy (Figs. 8.1 and 8.2) or cerebrospinal fluid) have permitted examinations of large numbers of cells (Ishihara, 1959; Sasaki, 1961; Goodlin, 1962; Grouchy *et al.*, 19631; Spriggs and Boddington, 1963; Ishihara

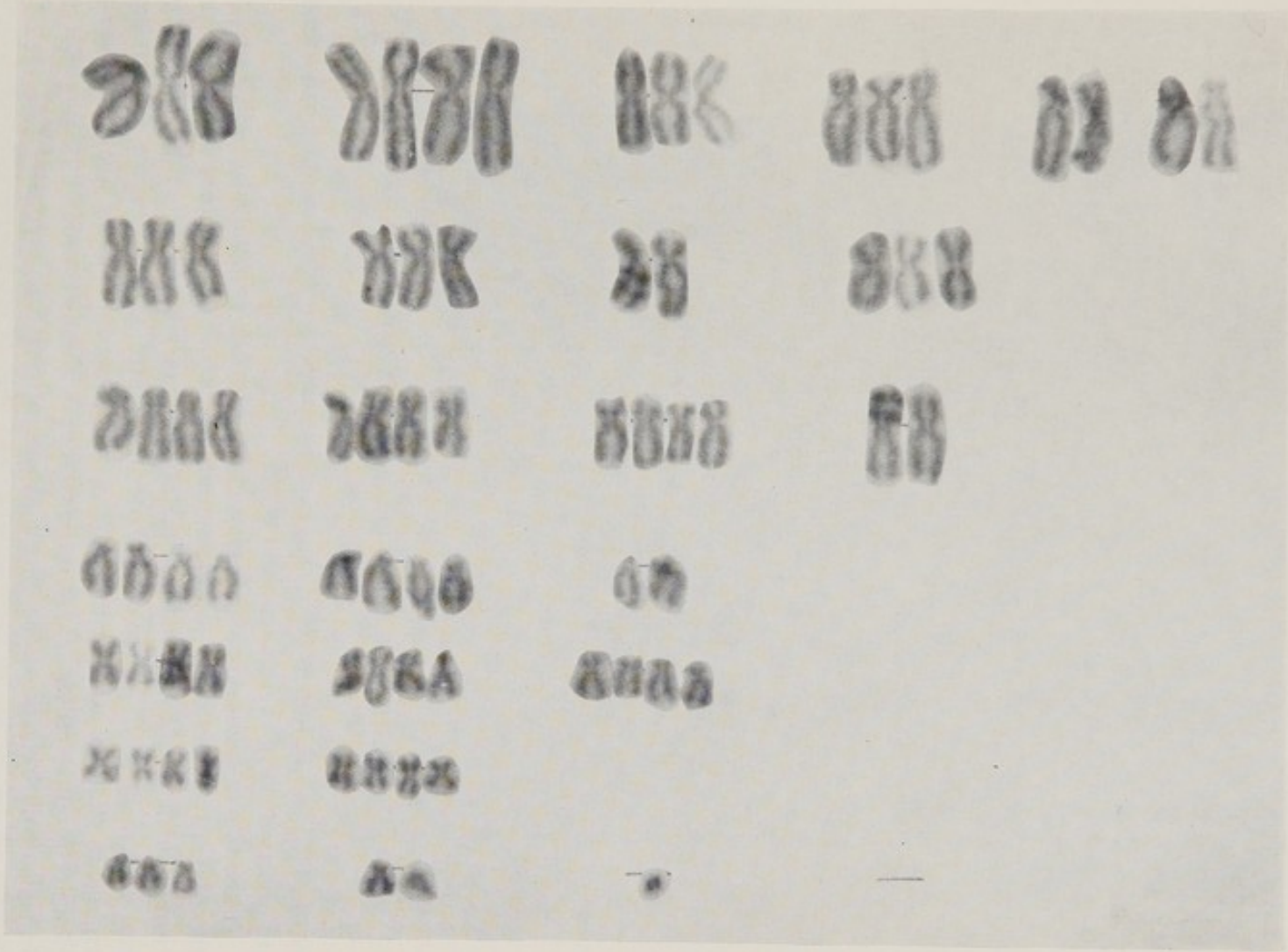


FIG. 8.4. Karyotype of a cerebral tumour (Institut de Progénèse, No. 800a).

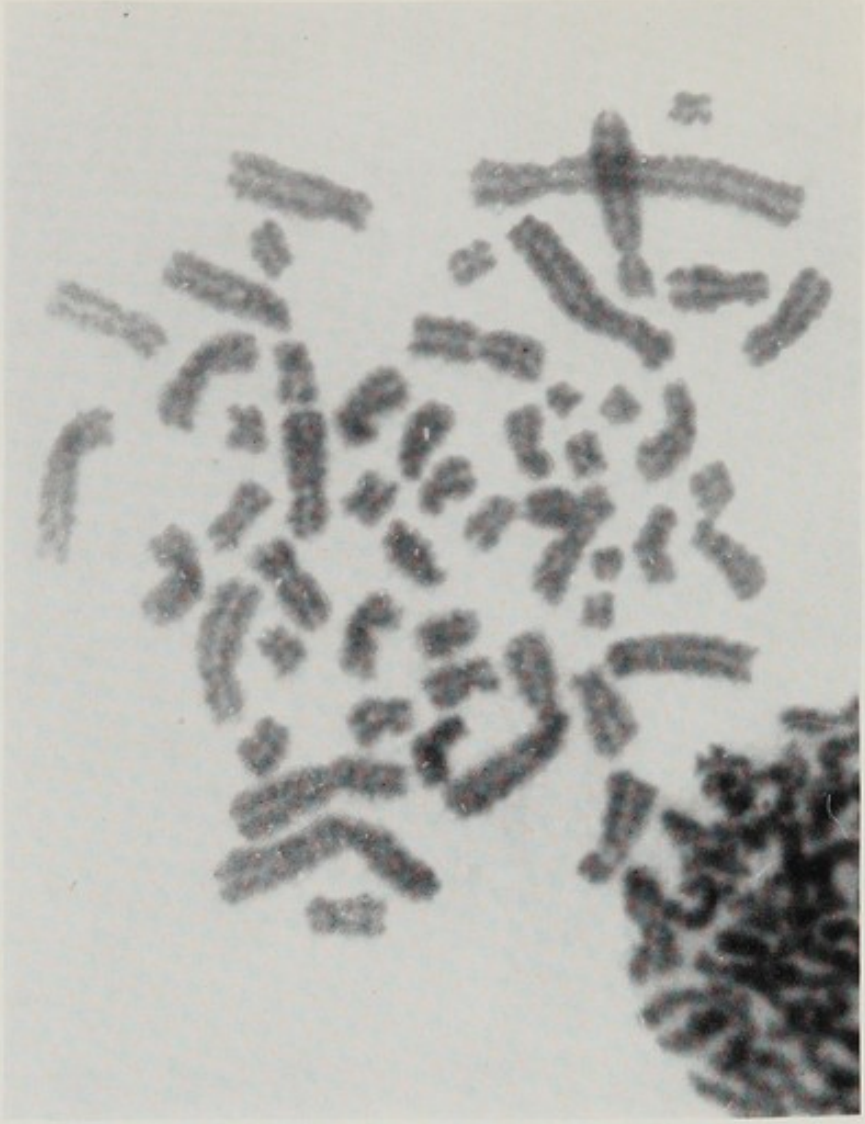


FIG. 8.5. Cell from an ovarian tumour (Institut de Progénèse, No. 1050).

et al., 1963). G. F. Jacobs (1961) has even proposed a method of diagnosis based on observation of these aneuploid cells.

This state of affairs springs from a major obstacle encountered in this study, the difficulty of culturing solid tumours. The various techniques of trypsinization or suspension by mechanical methods are very inadequate if the tumour is not extremely soft. Moreover, longterm cultures seem to reflect a complete change in selective equilibrium. After a very short time, a few weeks, the aneuploid cells tend to disappear and the culture is usually invaded by a population of cells of fibroblastic appearance and with normal karyotype.

This reversion *in vitro* of the selective value of neoplastic cells raises very many problems and its study is of great interest. At present this phenomenon considerably restricts the possibilities of investigation with routine techniques.

It is very difficult to give an overall idea of the results already obtained since the publications mentioned above concern tumours of very different origins although the majority of them are female sex tumours (breast, uterus and ovary).

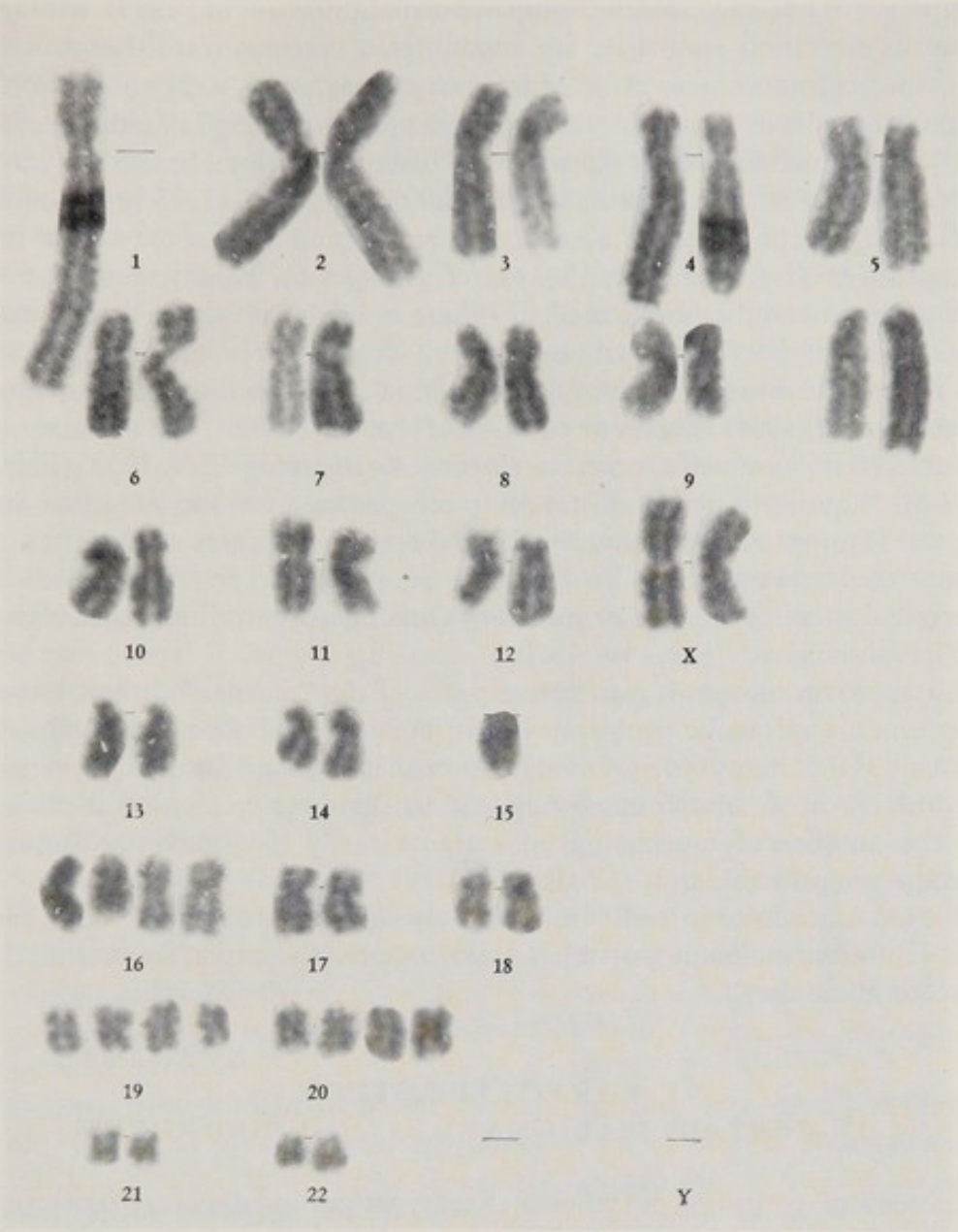


FIG. 8.6. Karyotype in a case of ovarian cancer. Note the two large acrocentrics below the pair No. 5 and the large marker chromosome placed in the position of the chromosomal pair 1. The other chromosome rearrangements vary from one cell to another (Institut de Progénèse, No. 1050).

Apart from the negative and inconclusive cases of Galton and Benirschke (1958) and Miles and Gallacher (1961c) the only general conclusion may be outlined as follows: benign tumours are accompanied by normal karyotype and DNA content (Stich *et al.*, 1960) whereas in malignant tumours the aneuploidies encountered range from loss of one or more chromosomes to the acquisition of a very large number of supernumeraries bringing the total to 50 or 60 or even 70 (Figs. 8.1 and 8.2), the polyploidy possibly being as high as 600 or 1000 chromosomes in an ascitic cancer of the colon (Sandberg *et al.*, 1963). Likewise

the amount of nuclear DNA is highly abnormal (Steele *et al.*, 1963). Moreover, numerous structural anomalies are encountered (various translocations, deletions, ring chromosomes, etc.). More particularly, very large submetacentric chromosomes have been reported (Ising and Levan, 1957; Grouchy *et al.*, 1963l). Reference has also been made on several occasions to large telocentric or quasitelocentric chromosomes much larger than the 13–15 group and resembling in morphology the abnormal chromosome 5 observed in the *cri du chat* syndrome (Lejeune *et al.*, 1963b), (cf. Chapter 6). This type of large telocentric was observed in duplicate by Ishihara in 1959 in a uterine carcinoma, by Ising and Levan (1957) in a lung tumour, by Grouchy *et al.* (1963) in the liquid of a degenerate ovarian cyst, and by Ishihara (1963) in three ovarian cancers and Auersperg (1964) in tumour cultures of the cervix uteri. We ourselves have encountered it in an ovarian tumour (Institut de Progénèse, No. 1050) (Figs. 8.3 and 8.4). A quite similar modification in morphology was reported in a Sternberg cell (Spriggs and Boddington, 1962a) and in five cases of Burkitt's lymphoma (of 9 cases studied) by Jacobs *et al.* (1963b). Tjio *et al.* (1963) have observed another type of supernumerary (small acrocentric) in a case of malignant lymphoma.

This apparent morphological convergence of the "marker" chromosomes of these tumours raises the problem of identification of abnormal chromosomes. It is obvious that many types of modification affecting the different chromosomes and differing in genetic content may lead to the same cytological picture and that the number of morphological variants easily identifiable with present techniques is probably quite small.

This identification can really be made only when a particularly simple karyotypic evolution makes it possible to reconstitute the probable origin of the observed anomalies.

C. KARYOTYPE STUDY IN CERTAIN MALIGNANT BLOOD DISORDERS

A rational classification of malignant haemopathies and diseases of the reticuloendothelial system cannot yet be based on the chromosome aberrations which may accompany them.

It is therefore practical to present the various disorders which have been the subject of karyotype study by simply arranging them under the usual headings. The disorders in which no anomaly has been detected (Vaquez's disease, for example, Traczyk [1963]) will not be mentioned here.

Waldenström's macroglobulinaemia

This blood disease characterized by presence of serum macroglobulin is classified here as malignant because of the frequency with which it progresses towards malignancy.

Few cases have so far been published (five in all, but not all equally definite). Bottura, Ferrari and Veiga (1961b) were the first to draw attention to the exist-

ence of a bone marrow clone with 47 chromosomes in a 71-year-old patient with macroglobulinaemia.

Of 90 cells, 49 had 47 chromosomes and 44 of these cells owed this abnormal number to the presence of a large chromosome the size of a 2 but much more distal than the latter. German *et al.* (1961) confirmed this observation on examination of a 32-year-old patient in whom they found a large extra mediocentric chromosome, this time found in 4 cells out of 90 obtained from circulating blood. Examination of the marrow of this patient enabled the authors to count six, technically imperfect, cells probably including 4 with 46 and 2 with 47.

Benirschke *et al.* (1962c) who also examined the circulating blood of a 69-year-old patient found 11 of 48 cells with 47 chromosomes including an extra large metacentric morphologically similar to that described by German *et al.*

Pfeiffer *et al.* (1962d) in a 63-year-old female found three cells with 47 chromosomes with a large submediocentric extra chromosome alongside 180 cells with 46 chromosomes, 9 with 45 and 8 with 44. However, it should be noted that in 12 of these cells with 46 chromosomes and one with 45 a large submetacentric was present larger than the normal chromosome 2, which led these authors to conclude that partial trisomy is possible. In the last case, a young female aged 22 showed two of 50 cells to have 47 chromosomes but no abnormal ones.

Finally, Elves and Israels (1963a) observed a very large extra chromosome associated with macroglobulinaemia (three cells of 160) and also the presence of an extra metacentric chromosome in two patients with moderate hypergammaglobulinaemia.

The fundamental proposition that Waldenström's macroglobulinaemia is due to the presence of a clone with 47 chromosomes, the supernumerary of which is a large submetacentric or subacrocentric remains to be demonstrated. The final conclusion will rest on the accumulation of new corroborative findings and on the demonstration, still to be made, of macroglobulin production by cells with abnormal karyotype.

On the other hand, it does not appear necessary to regard the morphological differences between the supernumerary chromosomes observed as irreducible. In fact, as Patau (1961) previously pointed out, various processes of recombination all leading to trisomy for a particular segment of chromosome 2 (?) could account for these apparent contradictions.

Multiple myeloma

Bottura and Ferrari (1962b) reported three cases with a normal karyotype and a fourth with α_2-globulin characterized by a clone with 45 chromosomes (16 cells with 46 chromosomes and 34 cells with 45 chromosomes) through loss of one of the small acrocentrics.

Castoldi *et al.* (1963) observed a clone with 44 chromosomes through loss of one of the 13–15 group and characterized by the presence of an abnormal medium-sized metacentric. Finally, Lewis *et al.* (1963a and 1963c) report three cases with clones ranging from 50 to 85 chromosomes. The latter appear to have a relatively simple development through progressive acquisition of supernumeraries (see below).

Hodgkin's disease

Spriggs and Boddington (1962a) observed aneuploidies ranging from 83 to 86 chromosomes in a lymph node displaying typical Sternberg cells. A variation of the same order was reported by Galan *et al.* (1963) who noted the presence of a very large chromosome (larger than 1) in aneuploid cells.

On the other hand, Ricci *et al.* (1962) reported only existence of regular tetraploids and diploids characterized by a medium-sized metacentric replacing a chromosome 17–18.

LEUKAEMIAS

As compared with other malignant disorders, leukaemias have been the subject of much more extensive study. Here, any generalization is still impossible and the use of the current diagnostic labels merely enables us to present these highly disparate findings. An exhaustive literature review was recently published by Berger (1964), and in this chapter we draw heavily on his work.

Chronic myeloid leukaemia

As early as 1960, C. E. Ford noted the existence of a chromosome fragment in a case of chronic myeloid leukaemia but the first studies of Baikie *et al.* (1960), although they eliminated the possibility of a bimodal distribution of the chromosome number, provided no confirmation for this observation.

It is to Nowell and Hungerford (1960e, c) that credit goes for having detected the presence of a very small acrocentric in the blood cells of two patients with chronic myeloid leukaemia, then in five others (Nowell and Hungerford, 1960d). Shortly afterwards, a new study of their patients enabled Baikie *et al.* (1960) to observe the presence of a small acrocentric in 8 of 12 patients with chronic myeloid leukaemia. The same group described these results in greater detail in 1961 (Toughe *et al.*, 1961). Other laboratories rapidly confirmed presence of the small acrocentric.

According to Berger (1964) analysing the observations of Adams *et al.* (1961), Bayreuther (1960), Bosi (1962), Fitzgerald *et al.* (1963a), Ford (1960a), Ford and Clarke (1963), Forteza (1962b), Hauschka *et al.* (1962a), Kinlough and Robson (1961), Nowell and Hungerford (1961–2), Ohno *et al.* (1961d), Reisman *et al.* (1963b), Sandberg *et al.* (1962a, b), Toughe *et al.* (1962a, 1963), Wahrman *et al.* (1962), Wahng *et al.* (1963) the literature mentions 147 cytological observations. Among these 147 cases the small acrocentric is found 127 times. However, it should be noted that certain negative results had been reported before the cultural behaviour of the cells carrying the anomaly was known. Nowell and Hungerford (1962c) showed that blood cultures fixed at 72 hr contained far fewer cells with the abnormal chromosomes than when the culture was fixed earlier at 48 hr. This phenomenon of differential survival of normal and abnormal cells may account for a number of examinations wrongly

interpreted as negative. Taken as a whole, these statistics, despite their imperfections, are highly suggestive and it may be considered even now that the very small acrocentric is the first known example of a *variant commun* the theoretical interest of which will be discussed in § D.

It should be noted that in the statistics of Toughe *et al.* (1963), uniform from the point of view of the quality of cytological observation, the small abnormal acrocentric was found in 25 of 27 patients.

Philadelphia or Ph¹ chromosome

This designation of the small acrocentric encountered in chronic granulocytic leukaemias, chromosome Ph¹, was proposed by British workers in line with the recommendation in the Denver document of naming abnormal chromosomes after the initial letters of the town of discovery (in the present case, Philadelphia).

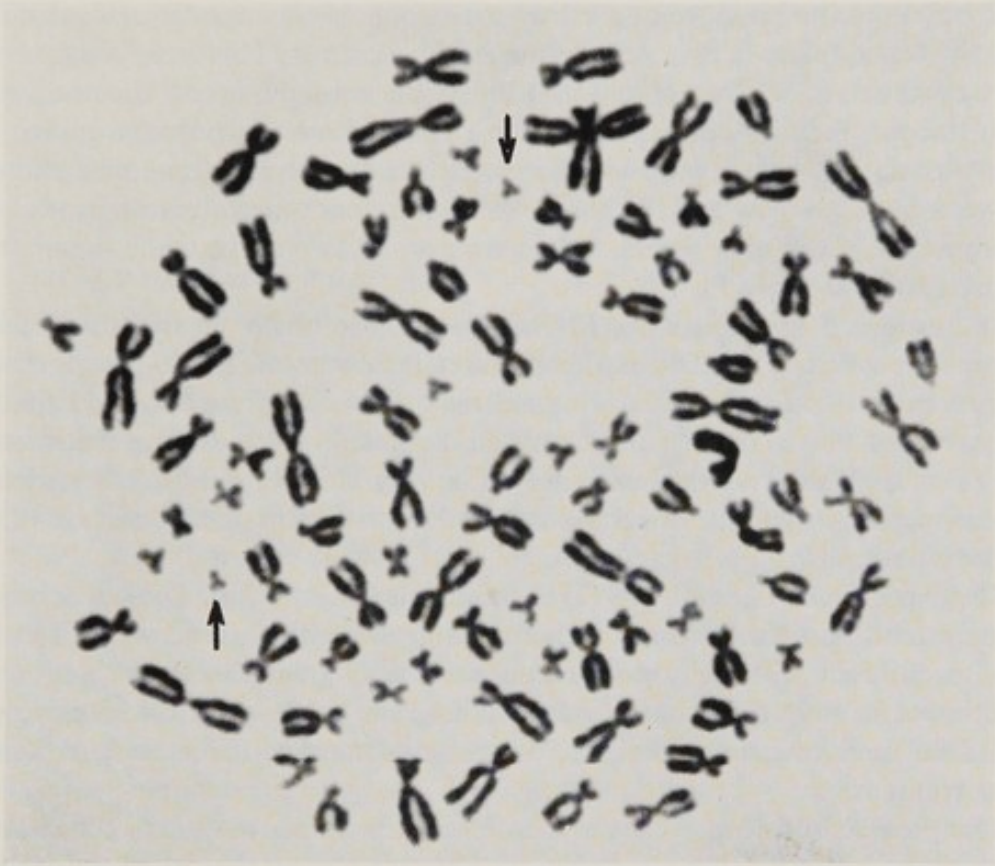


FIG. 8.7. Tetraploid cell in leukaemia with Ph¹. Note the two small acrocentrics of Ph¹ type (arrow). (Photograph by courtesy of Miss P. A. Jacobs.)

The morphology of the Ph¹ chromosome meticulously studied by Nowell and Hungerford, then by Toughe *et al.* (1961 and 1963), may be summed up as follows: this is a small acrocentric of the group (21–22) reduced by about half as compared with the size of a normal chromosome (Fig. 8.7). This reduction appears to be due to loss of the distal segment of the long arm although a more complex rearrangement cannot be ruled out. The presence of satellites has

frequently been noted on this chromosome and although identification is difficult, the majority of workers agree that this is a chromosome 21 showing partial deletion of the long arms. We shall see that although the morphology cannot provide a definite answer, the agreement of other findings, cytological as well as biochemical, suggests that this element actually derives from amputation of the chromosome which in triplicate gives trisomy 21.

The most important question then becomes that of defining the relation between the presence of a Philadelphia chromosome and the "leukaemic nature" of the cells which bear it.

Firstly, this chromosome is encountered only in the bone marrow or the blood of the patient; all other somatic cells are normal.

In the patients, the frequency of cells carrying Ph^1 is very revealing. According to Toughe *et al.* (1963) and Whang *et al.* (1963) practically all the bone marrow cells carry Ph^1 outside remissions or blastic episodes. Kemp *et al.* (1963b) even observed the presence of a Ph^1 chromosome in the marrow of a subject before the blood formula revealed the onset of leukaemia. This latter fact excludes the hypothesis of Schöyer (1963) that Ph^1 is the consequence of the disease.

In the peripheral blood it is common to encounter a variable proportion of cells with normal karyotype and others with a Ph^1 variant. A genuine dichotomy between bone marrow and the blood is very frequent especially in periods of remission: the blood only reveals cells with normal karyotype while the majority of marrow cells carry Ph^1.

According to Toughe *et al.* (1962a) the absence of Ph^1 in the blood cells is common if the number of cirulating white cells falls below 20,000 per mm^3 while its presence is constant in the bone marrow. These workers observed that the frequency of Ph^1 in the circulating blood dwindled on irradiating the spleen in six cases and showed the existence of a very clear correlation between the evolutive phase (activity or remission) and variation in the frequency of Ph^1 in the blood cells.

The experience of Levin *et al.* (1963) confirmed this notion. These workers demonstrated that transfusion of cells from subjects with chronic myeloid leukaemia to children with acute leukaemia previously treated with large doses of amethopterin and prednisone leads to a true graft. In these conditions peripheral blood cultures reveal the presence of chromosome Ph^1 for as long as 52 days after transfusion.

The presence of 60 per cent erythroid cells in marrow smears suggested to these authors that in chronic myeloid leukaemia the precursors of the red cells are themselves carriers of chromosome Ph^1. This notion is in full agreement with the observations of Toughe *et al.* (1963) and Whang *et al.* (1963): practically all the bone marrow cells are carriers of Ph^1 (Trujillo and Ohno, 1963).

The difference in the reaction of the red and white cells to the presence of a Ph^1 may not be so surprising as would appear at first sight. Cell differentiation implies a considerable difference in the metabolism of both lines. It is therefore plausible that certain genetic changes may have almost no effect on a cell line if the metabolism controlled by the changed genetic material is not directly implicated in differentiation of the line.

Finally, it must be pointed out that chromosome Ph^1 is only observed in cases of chronic myeloid leukaemia. In allied but clinically different proliferative myeloid conditions Nowell and Hungerford (1962a) in 10 cases and Sandberg (1962a) in 12 were not able to demonstrate a Ph^1 chromosome.

It may be considered that this abnormal chromosome is quite specific to chronic myeloid leukaemia and that it is present in the vast majority of bone marrow cells of patients thus constituting the *variant commun* of this condition.

Acute myeloblastic leukaemias

The present state of research into this type of leukaemia is far from displaying the unity and coherence of the observations made for chronic myeloid leukaemia. Various chromosomal anomalies have been reported but their diversity rules out any attempt at generalization.

It is quite possible that this apparent disorder corresponds to a biological reality, namely, that there exist different types of acute myeloblastic leukaemias not distinguishable from each other by classical haematological criteria.

Schematically, the observations may be ranked in three classes related to the anomaly observed.

(a) Loss of a small acrocentric

In 2 of 3 cases of acute myeloblastic leukaemia Ruffie and Lejeune (1962d) and Ruffie *et al.* (1963e) observed a line with 45 chromosomes through loss of one of the small acrocentrics which could not be a Y since this anomaly was observed in two males and one female. The same absence of a small acrocentric was then observed in cases of chronic myeloid leukaemia by Atkin and Taylor (1962), Toughe *et al.* (1963) and Speed and Lawler (quoted by Toughe *et al.* 1963). These authors say that the lost small acrocentric might have been a Y chromosome since only male subjects were involved here.

It is, however, tempting to bring together these observations and to consider the possibility of complete deletion of a chromosome 21 producing an even more serious lesion than that of the Philadelphia variant. This would lead us to assume a correlation between the course of the disease and the extent of the loss of chromosome material (Lejeune, 1962b).

In these conditions the observation of Forteza *et al.* (1962b) of a small so-called minute chromosome could be accommodated between the clones with 45 chromosomes through total loss of chromosome 21 and the clones with a classical Ph^1.

(b) Acquisition of extra chromosomes

Many observations in acute myeloblastic leukaemia and blastic transformation of a chronic myeloid leukaemia refer to the appearance of extra chromosomes.

Usually, the supernumerary is a medium-sized chromosome (6–12) which cannot be individually identified, at least in the majority of cases.

The first case of this type was reported by Ford (1960a) who observed a clone with 48 chromosomes, the supernumeraries being a small acrocentric and a medium-sized chromosome. The case of Kemp *et al.* (1961) described as XXY seems to correspond well to an additional acquired (6–12), the subject having no stigmata of the Klinefelter's syndrome. Of nine cases reported by Hungerford and Nowell (1962) five showed no anomaly, one displayed a clone with 45 chromosomes and three a clone with 47 through presence of an extra medium-sized one. The same observation of a medium-sized supernumerary was made by Weinstein and Weinstein (1963) and by Elves *et al.* (1963b). Finally, a supernumerary associated with the presence of Ph^1 was reported by Lüers *et al.* (1964b), Hammouda (1963) and by Levan *et al.* (1963), thus establishing the link between the Ph^1 isolated in chronic myeloid leukaemias and the medium-sized supernumeraries of acute myeloblastic leukaemias.

It would be tempting to conclude from these findings that there is a characteristic *variant commun* in acute myeloblastic leukaemias and that blastic transformation of the chronic myeloid leukaemias is accompanied by an identical *variant commun*. A medium-sized chromosome still not defined would be implicated in this unifying hypothesis but it is much too early to consider this possibility other than as a working hypothesis.

A tendency towards endomitosis is reported by Bottura and Ferrari (1963) and other observations refer to the appearance of many more numerous supernumeraries: Baikie *et al.* (1961c) observed 7; Bottura *et al.* (1961a) report variation ranging from 46 to 54 chromosomes; Ricci *et al.* (1962a) observed one large additional telocentric and one large submetacentric; Gavosto *et al.* (1963) mention karyotypes with 47, 48 and 49 chromosomes, the supernumeraries being (13–15), (16–18) or (19–20). These variations recall the preceding observation of Baikie *et al.* (1959) (karyotypes ranging from 46 to 50 chromosomes).

These few observations strongly suggest a clonal evolution through successive accumulation of anomalies as Ford and Clarke (1963) deduced from observation of karyotypes with 46, 47 and 48 chromosomes in a case of acute leukaemia. In another case, these same authors were able to follow in time the development of a mutant clone during a blastic transformation in a patient with chronic myeloid leukaemia. In the chronic phase the bone marrow cells of this patient displayed two markers, chromosome Ph^1 and a too large acrocentric 13–15; (this karyotype strongly resembles that of the patients of Ohno *et al.* [1961d] and Sandberg [1962b]).

After therapeutic remission, the patient during a relapse showed, in addition to Ph^1 and the large acrocentric, an extra medium-sized mediocentric as large as a 6 and two medium-sized chromosomes respectively replacing one missing chromosome 1 and one missing chromosome 16. Ford and Clarke observed during later examination the appearance of new variations and showed that they were related to each other by common anomalies. Although the treatment received by the patient might have falsified the pattern of this development, it is interesting to compare it with that described by Lejeune *et al.* (1963b) in a congenital leukoblastosis (cf. p. 152).

Other types of leukaemia

In 22 cases of chronic lymphoid leukaemias gathered from the literature by Berger (1964) no chromosome anomaly has been reported. The observation of Gunz *et al.* (1962) must be placed in a separate category. It concerns two brothers, both affected and both carrying an anomaly of one of the small acrocentrics (21–22). One of these chromosomes was amputated at the short arm and of horsehoe shape. This anomaly was found in three healthy members in six of this family. The relation between this anomaly and a "predisposition to neoplasia" is suggested by the fact that a sister of the patients had uterine cancer and that their father had died from a mediastinal sarcoma.

This failure to detect typical lesions in chronic lymphoid leukaemia contrasts with the observations made in cases of acute lymphoblastic leukaemia. Sandberg *et al.* (1961 a) emphasized the high frequency of aneuploidy, also reported by Pearson *et al.* (1963) in two monozygotic twins, both affected (cells with 64 chromosomes). Hungerford and Nowell (1961) observed various anomalies in 4 cases, and Grouchy and Lamy (1962) reported a case with partial deletion of a medium-sized chromosome (10) quite similar to one of our cases (Institut de Progénèse, No. 302).

The observations of acute or subacute myelomonocytic and monocytic leukaemias sometimes refer to aneuploidies (Baikie *et al.*, 1961 b) or only mention normal karyotypes (Kinlough and Robson, 1961 b; Campbell *et al.* 1962; Institut of Progénèse, No. 644). A case of Naegeli-type leukaemia (Institut de Progénèse, No. 450) was accompanied by presence in the bone marrow and also in a fascia biopsy of a small acrocentric very closely resembling a Ph^1 chromogene (Figs. 8.8 and 8.9). This recalls the observation of Gunz *et al.* (1962) quoted above. A relatively large number of leukaemias of different stem lines have been studied. Some have revealed hypoploidies (Ford *et al.*, 1968; Baikie *et al.*, 1961 b) or a tendency towards clonal development (Bottura *et al.*, 1961 a; Ford and Clarke, 1963, already mentioned). A definite aneuploidy was noted by Reisman *et al.* (1964) in all of seven cases. Finally, Fitzgerald *et al.* (1964) claim that chromosome lesions were found in bone marrow cells eleven times in eleven cases as against only three times in the eleven observations for the blood.

Leukaemia in patients with trisomy 21

It is probably artificial to arrange leukaemias among patients with trisomy 21 in a single uniform group since among these patients we find myeloblastic, lymphoblastic or stem-cell leukaemias. The only common feature is that usually they constitute an acute process.

Dysregulation of granulopoiesis has been mentioned in trisomy 21 by Ross *et al.* (1962) who gathered four cases simulating congenital leukaemia with spontaneous remission of the haematological syndrome.

Among the observations of confirmed leukaemias some refer to no anomaly apart from constitutional trisomy 21 (Toughe *et al.*, 1961; Brown and Propp,

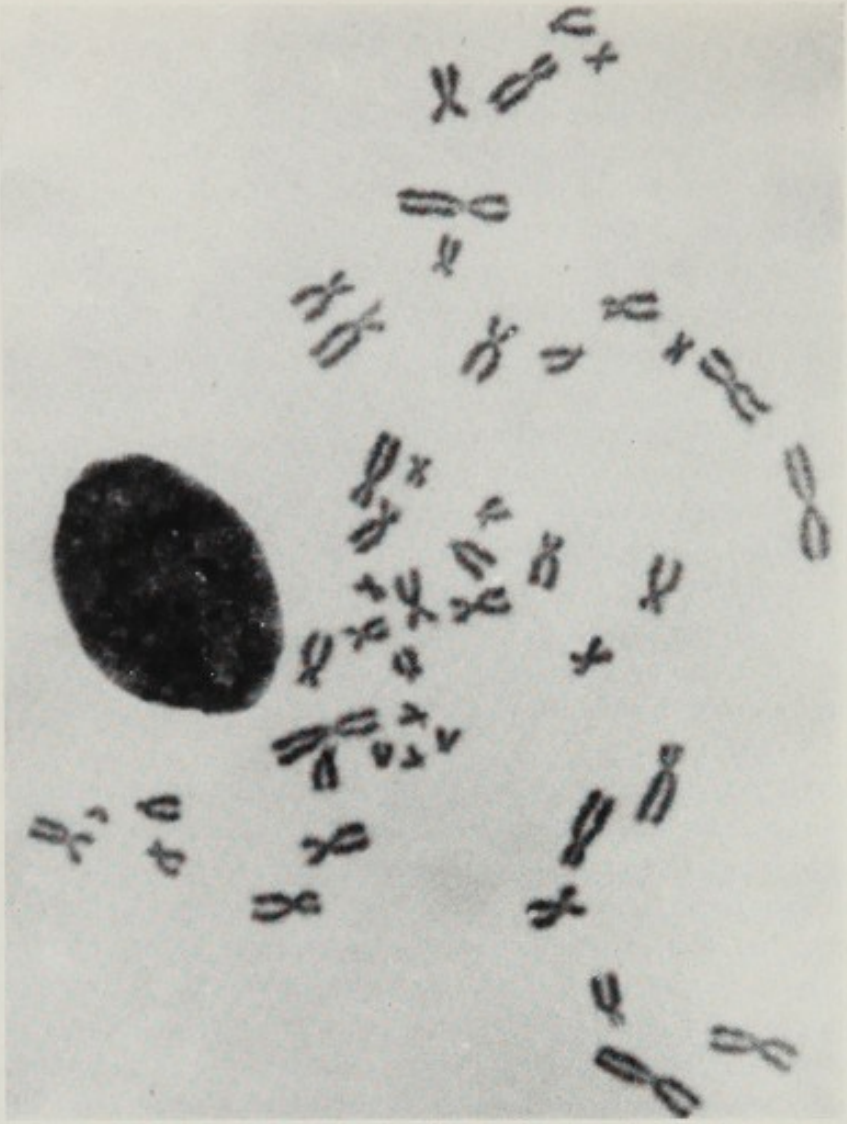


FIG. 8.8. Cell in a case of leukaemia with chromosome Ph^1 (Institut de Progénèse, No. 450).

1962; Reisman *et al.*, 1964, in one case in three) which may be masked by a translocation (German *et al.*, 1962d). In the light of the difficulties of culturing neoplastic as compared with normal cells, it is difficult to assert that no anomaly was actually present in these apparently negative cases.

Occurrence of extra chromosomes

Johnston (1961a) in a case of acute lymphoblastic leukaemia and Warkany *et al.* (1963a) and Reisman *et al.* (1964) each in a case of acute myeloblastosis have reported the presence of an extra medium-sized chromosome (6–12) in a number of cells. The frequency of the cells with 48 seemed to follow the course of the disease (disappearance during remission) in the case of Warkany *et al.* 1963a).

More complex anomalies (cells with 49 chromosomes including trisomy 21 but combining a monosomy 19 or 20 and presence of an extra medium-sized

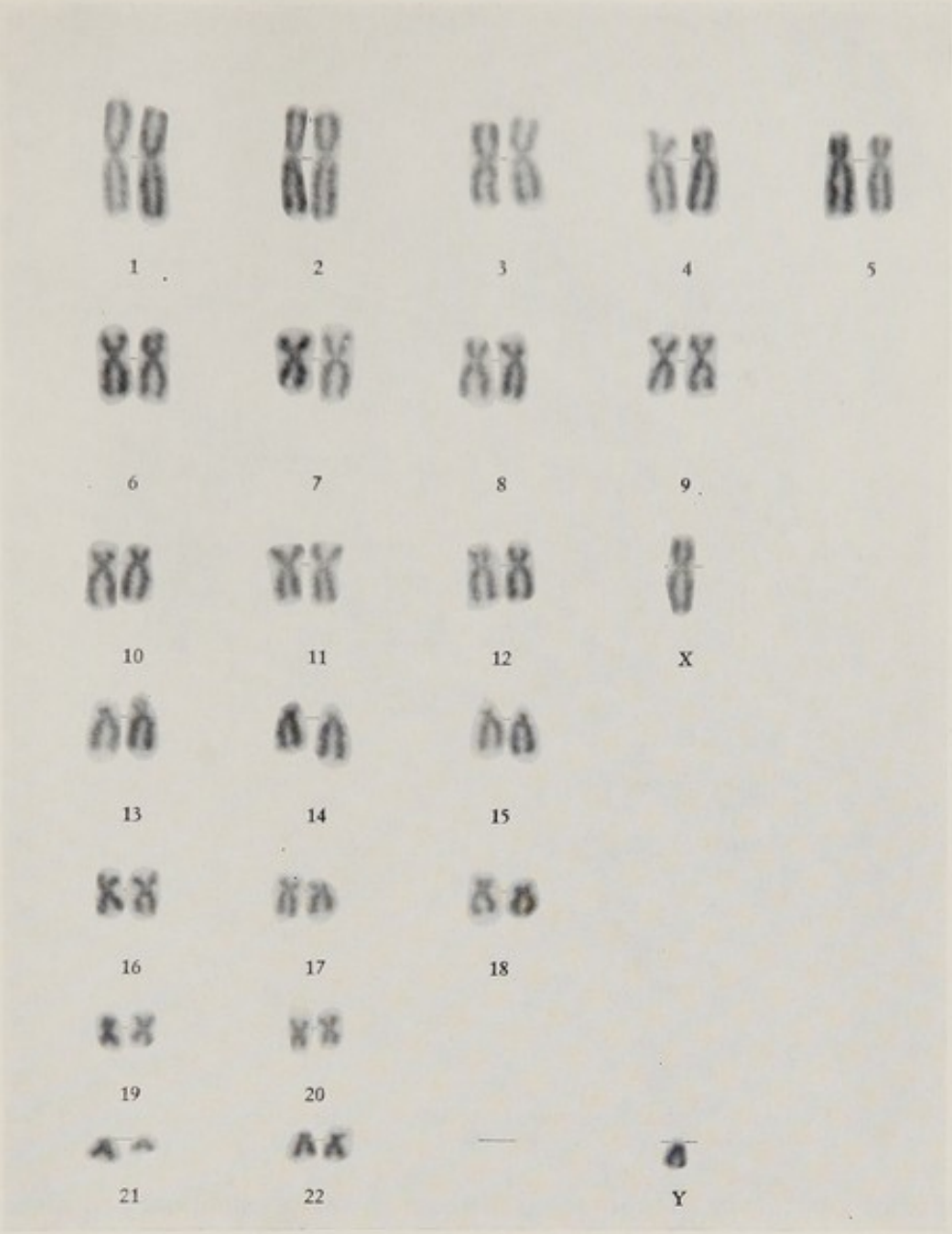


FIG. 8.9. Karyotype in a case of leukaemia with chromosome Ph[1] (Institut de Progénèse, No. 450). Note the difference in size of the two 21 chromosomes.

chromosome (6–12) and two metacentric chromosomes resembling a chromosome 3) are reported in a 2-year-old trisomic 21 with acute myeloblastosis by Vincent *et al.* (1963).

A very important numerical variation is mentioned by Sandberg *et al.* (1961 a) in two cases, one ranging from 46 to 51 chromosomes in acute lymphoblastosis in a 19-year-old trisomic 21, the other from 45 to 85 in acute myeloblastosis in a newborn infant. Likewise, Haylock and Williams (1963c) found anomalies superimposed on trisomy 21.

Because of the small number of cells examined in these various cases and the

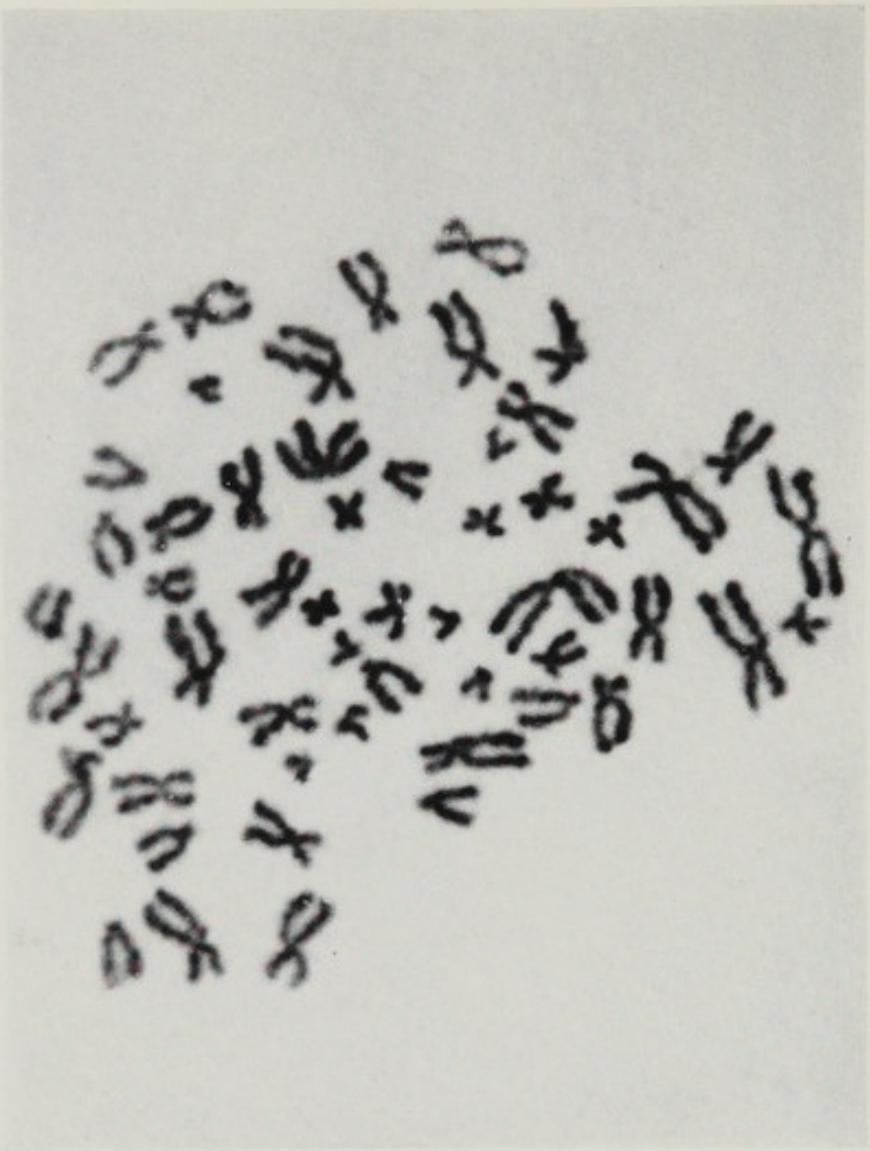


FIG. 8.10. Cell with 54 chromosomes. Leukoblastosis in a subject with trisomy 21.

absence of karytotype data in some of them it was possible only to discern a tendency towards hyperploidy through appearance of supernumeraries, the most constant of which appears to be a medium-sized chromosome (group 6–12).

Pattern of clonal evolution of the karyotype

From analysis of 80 karyotypes, a case of congenital leukoblastosis (Lejeune *et al.*, 1963) revealed a very characteristic clonal evolution which represents the only example in which all the intermediaries between the basic karyotype and the majority clone with 54 chromosomes (Figs. 8.10 and 8.11) have been identified.

This was a little girl who from birth showed erythroblastic then leukoblastic growth which spontaneously regressed. A picture of acute leukoblastosis progressively developed through successive advances culminating in a clinical picture of typical leukaemia at the age of 2 leading to death at the age of $2\frac{1}{2}$.

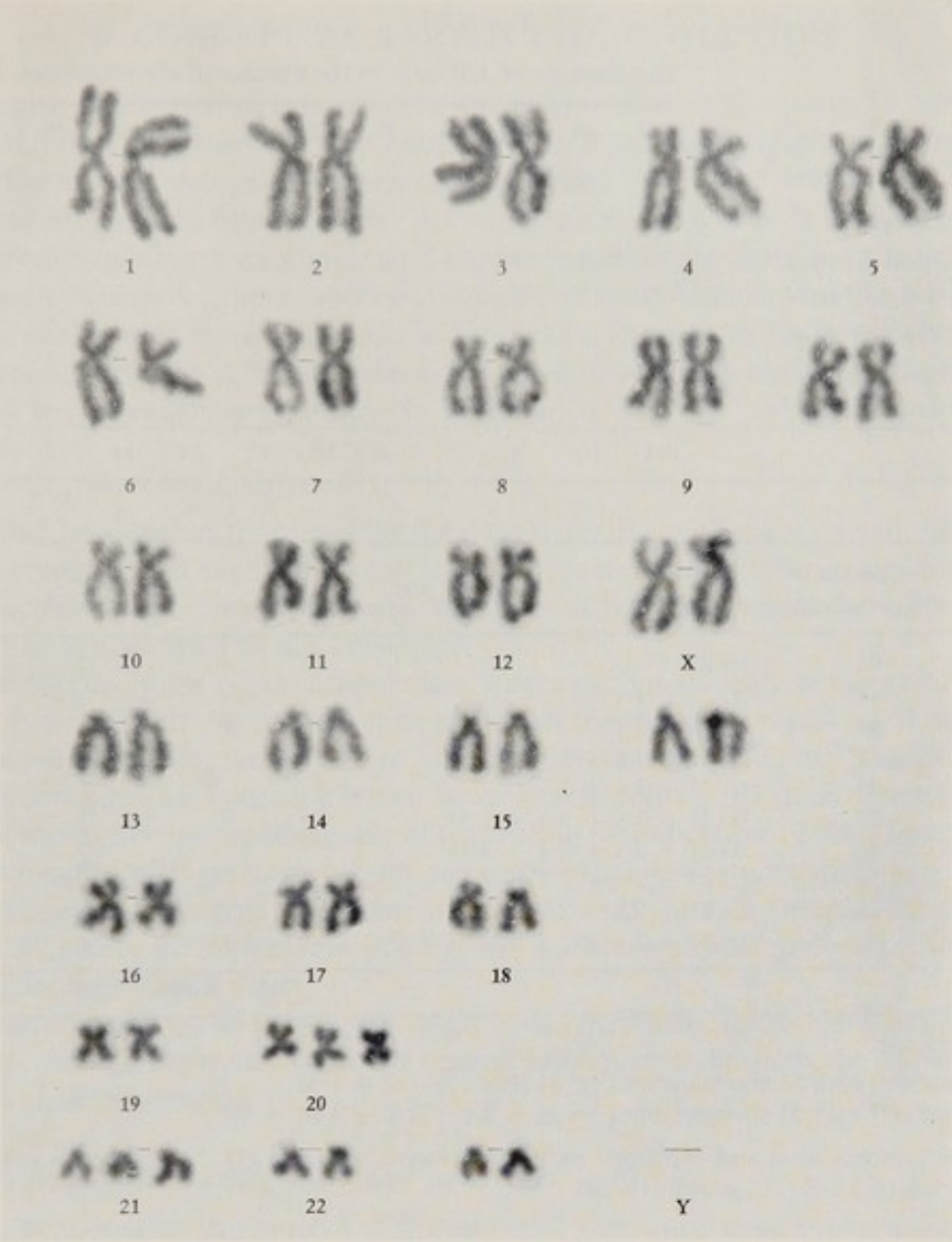


FIG. 8.11. Karyotype with 54 chromosomes. Note the presence in duplicate of supernumeraries.

A skin biopsy showed a classical trisomy 21 karyotype.

Three months before death and before any treatment, two successive examinations showed the numerical variations listed in Table 8.1.

Karyotypic analysis of 80 cells helped to established the morphological diagnosis of the excess elements.

The progressive accumulation of anomalies and diploidization of the extra chromosomes are the two main characteristics of this course.

It is well worth noting that the few karyotypes observed by Biedler and Murphy (1963) in an acute lymphoblastic leukaemia in a 12-year-old with trisomy

TABLE 8.1

Distribution of 320 cells by the number of chromosomes

	47.	48.	49.	50.	51.	52.	53.	54.	55.	56.	Total
Blood (first culture)	6	1	2	–	–	1	–	1	–	–	11
Bone marrow											
Liquid medium	8	–	1	3	4	6	12	36	8	2	80
Solid medium	109	9	1	–	–	–	–	3	–	–	122
Blood (2nd culture)											
Fixation at 48 hr	42	–	–	3	–	3	–	25	3	–	76
Fixation at 68 hr	26	–	–	1	–	–	–	4	–	–	31
Total	191	10	4	7	4	10	12	69	11	2	320

Number of chromosomes	Karyotype	Number of cells
47	tri 21	22
48	tri 21 + 1 v	3
49	tri 21 + 2 v	1
50	tri 21 + 2 v + 1 T	1
51	tri 21 + 2 v + 2 T	1
52	tri 21 + 2 v + 2 T + 1 M	3
53	tri 21 + 2 v + 2 T + 2 M	7
54	tri 21 + 2 v + 2 T + 2 M + 1 C	36
55	tri 21 + 2 v + 2 T + 2 M + 2 C	3
62	tri 21 + 2 v + 2 T + 2 M + 2 C + 18 + 5 M + 2	1
	Total	78

Exceptions:

one cell with 51 chromosomes tri 21 + 2 v + 1 T + 1 M;
one cell with 53 chromosomes tri 21 + 2 v + 2 T + 1 M + 1 C.

The symbols used and explained earlier represent: v, group (21–22); T, group (13–15); M, group (6–12–X); C, group (19–20).

21 resemble this evolution (karyotype ranging from 47 to 51 chromosomes, the supernumeraries being for the karyotype with 51, a v, two T's and an M).

Kiossoglou *et al.* (1963) observed a variation ranging from 47 to 51 chromosomes in a case of acute myeloblastic leukaemia in a 4-year-old trisomic 21 boy with a normal twin sister. Here, too, the appearance of small extra acrocentrics (21, 22), medium-sized acrocentrics (13–15) and small metacentrics (19–20) is akin to the karyotypic development described above. Finally, Reisman *et al.* (1964) report a karyotype with 55 chromosomes quite similar to those in a case of stem line leukaemia in a trisomic 21.

D. CONCEPT OF KARYOTYPIC EVOLUTION

In spite of absence of experimental confirmation of this concept in human solid tumours it is possible to schematize this hypothesis rapidly in order to show the aims of cytogenetic research in this field.

If one attempts to bring together the two phenomena so clearly associated in practice, neoplastic development and chromosome aberrations, it is possible to imagine a relatively simple model but capable of assuming many modes.

One of the most immediate tasks in this field is to see whether this evolution corresponds to certain "laws" which still have to be discovered but for which several hypotheses may even now be proposed.

(1) Progressive clonal evolution

It may be assumed that between the neoplastic line with a completely modified karyotype and the normal cell there is a succession of a large number of intermediate stages which through acquisition of successive anomalies have led to the final upheavals of the karyotype.

The succession of these intermediate stages cannot in practice be observed but it is tempting to reconstruct "the history of the clone" by assuming that the least aneuploid cells, the nearest to normal, represent the relic, the "fossils" of this evolution. This approach which scarcely differs from the usual practice of palaeontologists has the advantage of permitting identification of the abnormal chromosomes with greater or lesser certainty. When the latter appear in a still little changed karyotype it is sometimes possible by simple comparison with other elements of normal morphology to deduce which simple modification could have produced them.

From comparison of karyotypes of growing complexity it is sometimes possible to arrange them and to find a certain hidden logic beneath the apparent chaos of the karyotype.

(2) Tendency towards duplication of supernumeraries

It is possible that this pattern of chromosome aberration does not come about strictly by chance but there are certain tendencies or even certain laws governing this evolution. It seems, for example, possible that the tendency towards duplication of extra, normal or abnormal chromosomes is one of the most frequently encountered evolutionary modes (Lejeune, 1963 e).

The presence in duplicate of normal or rearranged extra chromosomes has in fact been observed on many occasions in human neoplasia (Ising and Levan, 1957; Ishihara, 1959; Sasaki, 1951; Spriggs and Boddington, 1962a, b; Lejeune *et al.*, 19631; Grouchy *et al.*, 19611 and m; Biedler and Murphy, 1963; Kissogolou *et al.*, 1963). The same observation has been made by Nichols (1963) in the karyotypic development of Roux sarcoma and by Auersperg (1964) in cultures of cervix uteri tumour. More generally, the extra chromosomes of plants known as accessory chromosomes (Muntzing, 1958 and 1963) show a tendency towards systematic duplication.

Certain deletions or rearrangements may be more probable than others and thus contribute to the variation of the karyotype.

(3) Hypothesis of the "variant commun"

The concept of the karyotypic development of a clone directed and corrected by the stringent selection operating *in vivo* against aberrant cells suggests the possibility of observing a further aspect of the picture. If the karyotypic change is not a pure epiphenomenon in neoplasia and if there exists a physiological relation between the properties of malignant cells and their chromosome contents it ought to be possible to detect in cancers of a given type a characteristic chromosome lesion which may be called the *variant commun*. As we have previously seen, the chromosome Ph^1 in chronic granulocytic leukaemia is the first known example of a *variant commun*.

The characteristics of a *variant commun* would then be the resultant of numerous mechanisms which remain to be discovered, such as the greater fragility of certain chromosomes, the more or less probable pathways of evolution and also the type of selection which probably differs from one tissue to another and may perhaps be strongly influenced by the physiological state of the organ or the whole body.

It seems plausible, however, that an intense karyotypic development producing serious aneuploidies and aneumorphies may mask this convergence by the accumulation of changes relatively random or at least peculiar to the tumour studied. Here, too, the *variant commun*, if it exists, can only be sought by attempting to reconstruct the history of the clones. Nor can it be excluded that hyperploidy confers on the clone a more flexible evolutionary potential and consequently a greater chance of "taking" (Haushka and Levan, 1953b).

Another phenomenon, somatic hybridization *in vitro*, studied by Barski *et al.* (1960a, b), Sorieul and Ephrussi (1961), Barski and Cornefert (1962) and Gershon and Sachs (1963), may greatly accelerate the rapidity of appearance of many possible genomic constitutions.

(4) Hypothesis of "combinaisons interdites"

Another goal of analysis would be to define possible *combinaisons interdites* (forbidden combinations). It is probable that disruption of the genetic material cannot be perpetuated in complete disorder. The biochemical reactions governed by the genes probably cannot be modified quantitatively in an entirely incoherent manner otherwise the cell would die.

To schematize this idea we may consider a reaction chain of the type

$$\begin{array}{c} A \rightarrow B \rightarrow C \\ \downarrow \\ D \end{array}$$

in which A is a substance present in limited amount, C, a substance essential for the life of the cells and D, another metabolic pathway of the intermediate B.

If there exists a quantitative relation between enzymatic activities and the number of chromosomes which govern them (cf. Chapter 15) it may be imagined

that excessive degradation of B to D (through appearance of an extra chromosome carrying the corresponding gene) cannot be viable at cell level if the available amount of A is not itself increased. In the last analysis duplication of the chromosome bearing the gene controlling the transformation of B to D can intervene *only after* the duplication of the chromosome bearing the gene controlling the production of A or the efficiency of its transformation into B.

Another mechanism of prohibition consists in the need for the clone not to develop antigenic qualities likely to be recognized by the host and leading to formation of effective antibodies. In this context it is interesting to note that all known cases of qualitative or quantitative variations in the blood groups in leukaemias (Salmon, 1964) produce only combinations "permitted" by the genetic constitution of the subject.

(5) Establishment of factorial maps

These still purely theoretical considerations lead us to look at karyotypic study of tumours from a new angle: analysis of the genetic content of certain chromosomes. Apart from possible *combinaisons interdites* neoplasias produce very many genomic constitutions infinitely more varied than those permitted in embryonic development.

The hope that finer analysis may make use of these variations to localize certain functions on certain chromosomes is justified by some results already obtained especially with regard to chromosome 21 (cf. Chapter 15).

In the experimental field Fjelde (1962), for example, observed a correlation between the appearance of certain markers and changes in serum proteins in mouse transmissible leukaemias (plasma cell). Likewise, Schreker *et al.* (1963) observed an association between the rise in dihydrofolate reductase and loss of a subtelocentric chromosome in the sublines of leukaemia L 1210. More generally, the level of proteins in seeds is proportional to the number of accessory chromosomes according to Fröst (1963). These findings may be likened to the observations made in man in Waldenström macroglobulinaemia and multiple myeloma.

It would not be surprising if karyotypic analysis of certain mutant clones proved to be a valuable aid in establishing factorial maps in man. In this event these maps, even relatively crude, would assume crucial importance. In fact, if the scheme of the *combinaisons interdites* represents a part of reality, a partial deficiency of substance A (through an antimetabolite or diet) would be more harmful for the mutant cell than for the normal cell. Seen in this way a karyotypic analysis of a tumour would have as its corollary the detection of the "sensitive" metabolism of the mutant clone.

To conclude this discussion on a rapidly developing and constantly changing theme it is interesting to compare the previous points with certain lessons drawn from experimental carcinogenesis.

The oncogenic action of viruses amply demonstrated in *anima vili* in no way contradicts the hypothesis of karyotypic evolution. In fact, certain viruses are capable of producing chromosome aberrations.

For example, infections by common viruses such as that of herpes (Hampar

and Ellison, 1961), and perhaps that of measles in man (Nichols *et al.*, 1962b), are capable of producing chromosome breaks and rearrangements. This latter point was not found by Tanzer *et al.* (1963). To be more precise the action of certain viruses such as SV 40 produces numerous modifications in tissue culture. There would appear to be preferential aneuploidy of certain chromosome pairs, the small and medium-sized acrocentrics, in particular (Koprowski *et al.*, 1962 and 1963; Shein and Enders, 1962a; Yerganian, 1962a,b; Moorhead and Saksela, 1963). Chromosome rearrangements have also been observed in Roux sarcoma (Levan, 1963a).

It may well be that the oncogenic role of certain viruses though not at present demonstrated in man (Trentin *et al.*, 1962; MacAllister and Hammon, 1963; Lepine, 1963) is based on a mechanism rendering chromosome mechanics more-fragile (Nichols, 1963). This may account for the results reported by Stoker (1963) on the radiation susceptibility of cells infected with polyoma virus.

Since the most obvious cellular effect of carcinogens, ionizing radiations, chemical agents (Biesele, 1958) and viral disorders is represented by the appearance of chromosome aberrations (simple ageing of the individual also promotes them, Jacobs *et al.*, 1963a) it becomes impossible to evade the fundamental question: are these variations of the genome the cause or the consequence of cancer?

No formal answer is yet possible. Nevertheless, the cytogeneticist, witness of the genomic upheavals affecting neoplastic cells, gains the impression that the chromosomal aberrations are probably neither the cause nor the consequence of cancer, they are the neoplastic process itself.

CHAPTER 9

Numerical Gonosomal Aberrations

OVARIAN DYSGENESIS

A new classification of gonadal dysgenesis and states of intersex based on direct study of sex chromosomes is now in hand.

Varieties previously confused are being dissociated, others previously thought of as separate entities appear as a single common gonosomal type, others previously unknown are in the process of being identified.

This new classification is cytogenetic. It distinguishes numerical and structural anomalies with or without mosaicism.

This nosological recasting is no easy task for numerical anomalies chiefly because of mosaicism, a phenomenon which eludes complete investigation, and for structural anomalies mainly because of the present techniques which permit detection of only the most apparent of these aberrations.

This is true of both because of our imperfect knowledge of the maturation of the sex phenotype despite recent very valuable experimental evidence.

Research preliminaries

The discovery of chromosome determination of sex and its aberrations in heterogametic bisexual lower species hinted at the possible existence of a similar situation in any species subject to the same laws of reproduction.

The announcement in 1958 and 1959 by three different laboratories (Ford *et al.*, 1958b; Tjio and Puck, 1958a; Chu and Giles, 1959) of the possibility of identifying the human sex chromosomes X and Y (gonosomes) implicitly encouraged cytogenetic study of gonado-somatic dysgenesis and human intersex varieties.

In fact, there was good reason to believe that some of these diseases could well have at their origin gonosomal formulae similar to the *Drosophila* formulae XXY2A and X2A among others, with due allowance for the sex determinants X and Y peculiar to each species.

Such a belief derived in part from study in these diseases of the "sex chromatin" discovered in 1949 by Barr and Bertram. This heteropycnotic body appears in the normal state at interphase in the nuclei of somatic cells of the female but not of the male cells. Because of this difference the chromatin was at first considered as a combination of the heterochromatic segments of two X chromosomes. This interpretation did not hold sway for long for the reasons outlined elsewhere

(pp. 278 and 280) but whatever the position one may adopt in a discussion still not closed, it appears, in fact, that an interphase nucleus contains the same number of chromatin bodies as X chromosomes minus one.

Applied as early as 1953 (K.L.Moore *et al.*, 1953; Moore and Barr, 1955) in study of gonado-somatic dysgenesis, the chromatin criterion which became the cytological criterion of the X chromosome gave very suggestive results.

About 50–80 per cent of subjects with dysgenesis of the seminiferous tubule (Klinefelter's syndrome) had a chromatin body (Bradbury *et al.*, 1956; Plunkett and Barr, 1956; Nelson, 1956; Grumbach and Barr, 1958; Lenz *et al.*, 1959c) which suggested that they had two X chromosomes, despite the masculine phenotype.

About 80 per cent of subjects with ovarian dysgenesis (Turner's syndrome) had no chromatin body (Décourt *et al.*, 1954; Polani *et al.*, 1954, 1956; Wilkins *et al.*, 1954; Nelson, 1956; Lamy, 1957; Delzant, 1960) which suggested that they had at most only one X chromosome, despite their feminine phenotype.

The subsequent detection of other gonosomal aberrations in number or even in structure from the pointers of the chromatin body confirmed and increased the value of this criterion. Anomalies in institutional centres were found among slighter forms of mental backwardness more than among severe forms (Gustavson and Akesson, 1961).

The other purely clinical assumption was afforded by study of the distribution of a "marker" allele of the X chromosome in families of index patients with Turner's or Klinefelter's syndrome.

Blindness to red–green, a recessive X-linked character, affects about 8 per cent of males and 0·43–0·44 per cent of females of Indo-European origin (Waaler, 1927). If the subjects with Turner's syndrome have only one X they must give the masculine proportion of colour-blind persons since, like males they are hemizygotes (Polani *et al.*, 1956). On the other hand, the proportion among the subjects with Klinefelter's syndrome must be equivalent to the female proportion.

These assumptions were in part verified. Since then they have stimulated research with the aid of new X chromosome markers, the results of which are considered in the chapter on the mechanism of gonosomal aberrations.

I. Forms without mosaicism

Turner's syndrome

The history of this gonado-somatic dysgenesis generally known as Turner's syndrome (H.H.Turner, 1938) started with the initial discovery of ovarian agenesis coming up to date with anomaly of the chromosome sex determinants, via discovery of a hormonal reaction of the anterior pituitary to primary hypogonadism.

Despite the temporary interpretative error which linked the symptoms of the disease to the pituitary, this is a fine example of a study well directed from the start to the end.

The first anatomical description was made by J. B. Morgagni in 1749, with mention of the essential anatomical signs: female of short stature, genital infantilism and ovarian agenesis and even including a renal malformation.

"A female of about 66 years old, of stature below average but much too large to be included in the species of dwarfs..." having succumbed to peritonitis is the object of an anatomical study. The infantile arrangement of the external genital organs, the "very poorly developed nymphs", "the orifice of the vagina so narrow ... that it would not allow the largest of the four fingers to pass through" claim attention.

The author then notes the shortness and narrowness of the vagina and uterus, the presence of very thin round ligaments, wide Fallopian tubes and ligaments longer than usual owing to uterine hypoplasia. Then, after careful examination of the wide ligaments, adds: "I quite clearly recognize that this woman had absolutely no ovary nor even the slightest rudiments of these organs".

Continuing the abdominal exploration, Morgagni noted that "the state of the kidneys was such as seen when these vesicles are full of serosity..." and his description is very suggestive of polycystic kidneys.

For nearly two centuries nothing essential was added to Morgagni's description. In 1938, H. H. Turner collating seven purely clinical observations showed that women of small stature, infantile and with primary amenorrhoea may be distinguished by morphological features, the most suggestive of which are webbed neck and cubitus valgus. The coincidence of genital infantilism and stunted stature led him to suspect that pituitary insufficiency was at the origin of his cases. Subsequent hormonal investigations were, however, to show that this was primary gonado-somatic dysgenesis.

In 1942, the primacy of gonadal lesions was demonstrated by increase in urinary gonadotrophins (FSH): over 50 and even 100 mouse units in 24 hr (Varny *et al.*, 1942; Albright *et al.*, 1942). This observation, together with absence of normal ovaries (Wilkins and Fleichmann, 1944), eliminated the thesis of primary pituitary insufficiency.

Inspired and guided by the preliminary discoveries of the numerical and morphological features of human gonosomes and the distribution of the chromatin body in subjects with gonadal dysgenesis (Wilkins *et al.*, 1954) cytogenetic studies were undertaken in 1959.

Basing their arguments on the so-called masculine aspect of the chromatin some, even at that late date, assumed that the gonosomal constitution of Turner's syndrome was XY. Others considered that the X0 constitution or a mosaicism could also account for the chromatin type (Polani *et al.*, 1956; Danon and Sachs, 1957).

The subject who was instrumental in allowing Ford, Jones, Polani, de Almeida and Briggs to make this discovery on 4 April 1959 (1959c) was a typical example of the disease. The bone marrow technique enabled them to count 45 instead of 46 chromosomes. After some discussion arising from the difficulty

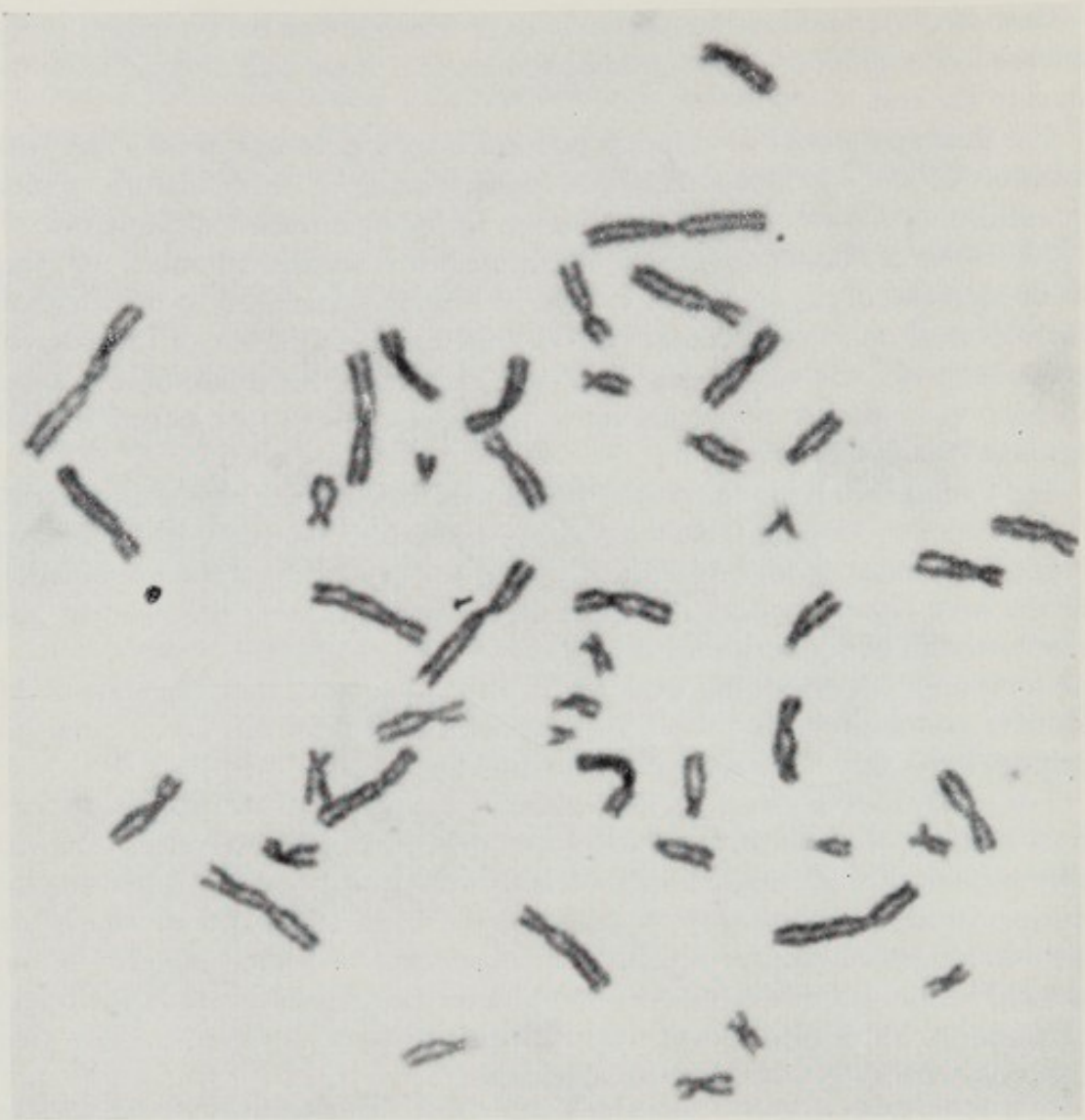


FIG. 9.1. X0 Turner's syndrome with 45 chromosomes.

of distinguishing the X chromosome from the 6, the authors considering that such autosomal haploidy would very probably be lethal concluded that a formula X0 was likely. Trisomy 21 was, in fact, already known and the absence of hapoidy 21 which might have been expected to occur with equal frequency was explained by the lethal consequences of absence of a chromosome 21.

This was rapidly confirmed by Jacobs and Stewart (1959), Fraccaro *et al.* (1959), Tjio *et al.* (1959), and others, using bone marrow techniques and on skin, fascia lata and blood.

But cytogenetic analysis, while multiplying the examples of the X0 variety in Turner's syndrome, isolated other karyotypic varieties.

Nosological consequences

From cytogenetic advances it is possible even today to attempt classification of pathological variants in this ill-defined mixed-up group known as Turner's syndrome.

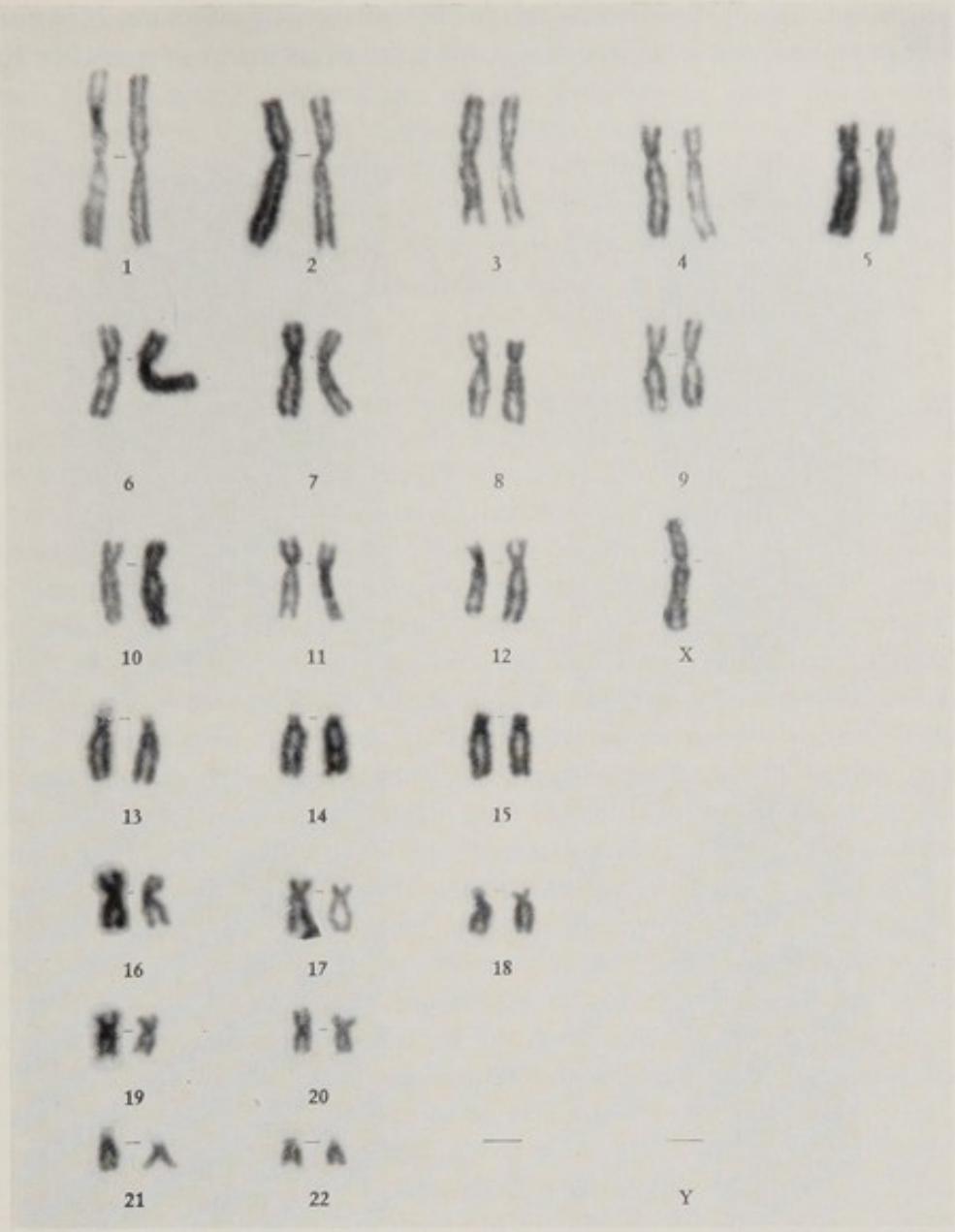


FIG. 9.2. X0 Turner's syndrome with 45 chromosomes.

This attempt leads us to distinguish, on the one hand, the X0 variety and to define some of its anatomo-clinical modes and to distinguish, on the other hand, more or less well known varieties with normal or pathological gonosomal formulae.

1. X0 Type

The X0 cell is distinguished both by absence of a chromosome assigned by morphology to group 6–12 X and by the absence of the chromatin body and heteropycnotic X detectable in the female by ^{3}H-thymidine. Often absence of drumsticks is also reported.

The X0 type being thus individualized by cytogenetic criterion, it is possible to attempt an analysis of its frequency, the conditions for its appearance and its features.

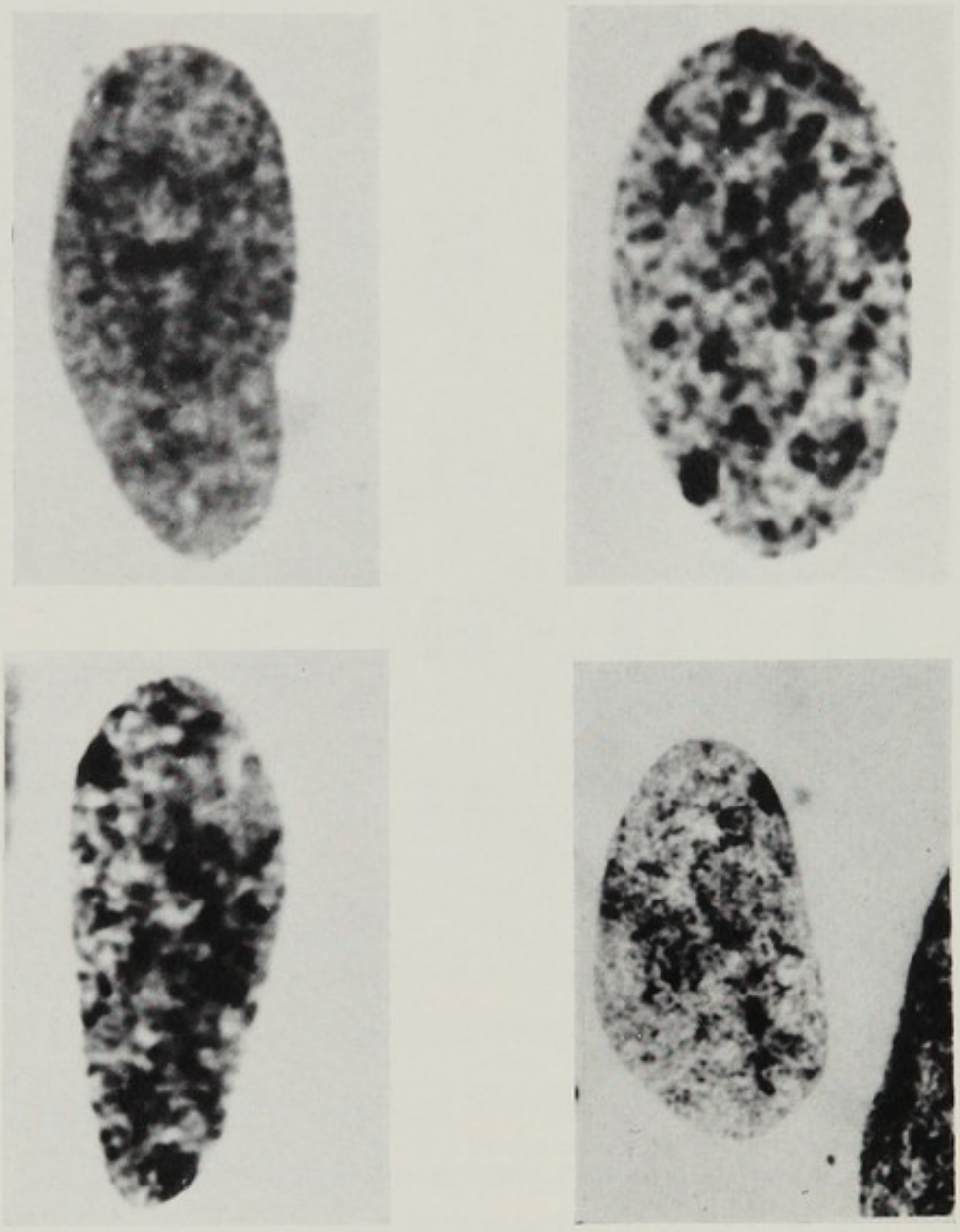


FIG. 9.3. Different types of nuclear chromatin. (a) Nucleus with no sex-chromatin. (b) Nucleus with single sex-chromatin mass. (c) Nucleus with two sex-chromatin masses. (d) Nucleus with three sex-chromatin masses.

(a) Frequency

The frequency of X0 types estimated from investigation of the chromatin criterion in 10,000 newborn females among births in Edinburgh and suburbs (MacLean *et al.*, 1964) was 0·4 per 1000. These figures when combined with the previous results obtained from K. L. Moore (1959) and Bergemann (1961 b) gave 5 negative chromatin subjects of 13,642 examined or 0·37 per 1000. This pro-

portion is lower than the possible theoretical value in relation to the frequency of XXY Klinefelter's syndrome at 2·1 per 1000 and in relation to that of XXX at 1·2 per 1000. Several explanations of this discrepancy have been proposed (p. 269). The inquiry made in Tokyo with the double criterion of height and chromatin body in 9166 schoolchildren 6–18 years gave a figure of about 0·4 per 1000, no doubt lower than the actual value since the inquiry covered only schoolchildren whose height was distinctly less than normal, and could not take into account X0 subjects who died before school age.

(b) Conditions for appearance

(i) Certain familial X0 peculiarities have been isolated. The influence of maternal age has been invoked with a bimodal tendency in the distribution (Penrose, 1961 b). More numerous documents dealing with X0 subjects and not only negative chromatin ones are still necessary to evaluate these debatable influences (W. Lenz, 1959, Part II; Boyer *et al.*, 1961; Lindsten, 1963a).

In fact, neither the age of the mother nor the father, nor the birth rank seem to influence the X0 frequency. And yet the proportion of aneuploid cells in cultures of leukocytes appears to increase with the age of the subject with increased risk of loss of X for the female or of Y for the male (Jacobs *et al.*, 1961 b, 1963a). Other facts such as the relatively low proportion of X0 sisters and frequency in these families of twin pregnancies seem sufficiently relevant to require verification (Lindsten, 1963a). The proportion in these families of normal twins of identical and different sexes agrees with the ratio expected.

The observations reported do not mention for the progeny of X0 mothers abnormal frequency of miscarriages or stillbirths. This fact too warrants further study, since expulsion of X0 eggs is demonstrated by certain karyotypic results (Carr, 1963) and because it may explain the relatively low number of viable X0 neonates in relation to the theoretical figure expected (p. 269).

(ii) The general phenomenon of familial coincidence of chromosome aberrations has been found in some rare cases of Turner's syndrome.

Before the use of the cytogenetic criteria, several examples of Klinefelter's and Turner's syndromes in the same sibship were reported (Bassoe, 1956).

Since then an example of trisomy 13 in a sib of an X0 girl has been published (Therman *et al.*, 1961); a case of trisomy 21 (Johnston, 1962); and another in the sib of a girl with gonadal aplasia and negative chromatin (Ferguson-Smith, 1962c).

The coincidence of Turner's syndrome and trisomy 21 was again observed in another family (Zellweger and Mikamo, 1961 b) consisting of a trisomic 21 girl, a haplo X girl and three normal sibs; and another X0 case in the progeny of a normal male who was the cousin (four times removed) of a boy and a girl both with trisomy 21.

The coincidence of Turner's X0 syndrome and a translocation 2 ~ G was observed in a family with 14 living subjects distributed over four generations (Lejeune *et al.*, 1963a). Ten of the 13 living relatives of the X0 subject were studied. The balanced translocations without apparent phenotypic anomaly gave a large acrocentric formed of a chromosome 22 and a large distal segment

of the large arm of a chromosome 2. This neochromosome was found in six relatives of the X0 patient; her mother, three of her four brothers and a nephew and a niece, children of her elder brother with translocation. Five of the ten pregnancies of the mother of the X0 patient had terminated in miscarriage (p. 130).

(d) Semeiology

(i) The small stature is constant, 1·45 m on average—sometimes less. The value of this sign was brought out by the inquiry mentioned above made in Tokyo schools (Nakagome *et al.*, 1963a). Among 9166 schoolchildren from 6 to 18 years, 268 were selected because of their small stature judged in relation to the standard curve (-2σ). Within this group 41 diverging even more from the mean (-3σ) were again selected. From these 268 children, in four subjects chromatin-negative Turner's syndrome was found, including three in the 41 group. This skeletal hypotrophy was accompanied by disturbances in development of the epiphyseal zones and more or less marked structural dysplasia.

1. The various epiphyses of the elbow joint are often affected, usually resulting in cubitus valgus, noted in half the cases. With an equal frequency other anomalies are noted in the bones of the shoulder and forearm with shortness or S deformation of these bones, in particular, of the radius.

The epiphyseal anomalies in the knee joint affect about two subjects in three, giving the medial tibial condyle an anvil appearance (Kosowicz, 1959).

Finally, the shortness of the metatarsals and metacarpals is a frequent feature. It is not an exaggeration to say that it is encountered in half the cases; it affects always the fourth metacarpal and the fourth metatarsal, sometimes also the third and fifth.

The rachis is less often affected. The sella turcica is sometimes small—vertebral blocks, kyphoscoliosis and anomalies of the sacrum (spinal distal hypoplasia) are a quite rare occurrence.

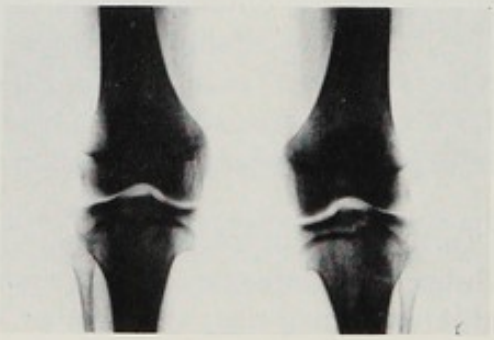


FIG. 9.4. Overhanging tibial plateau (Kosowicz's sign). X0 Turner's syndrome.

2. The second skeletal anomaly is structural dysplasia observed in about half the cases and often at an early stage. When slight, it is confined to the spinal cord and pelvis, when more intense it is diffuse. This term is preferred by certain workers to that of osteoporosis (Lindsten, 1963a). Disturbances in skeletal

development thus constitute a noteworthy aspect in X0 subjects. They help to give them their special habitus: small size, facial dysmorphia with hypertelorism and sometimes epicanthus, and facial asymetry and micrognathism. They are accompanied by increase in the plasma level of the growth hormone (Fraccaro *et al.*, 1960c). One subject in two is affected by webbed neck.

The shoulders are broad, neck short, nipples small and lateral and the thorax developed like a "shield". These anomalies are accompanied by cutis laxa with or without pigmented naevi and low growth of the hairline is frequent; there may be lentiginosis and, more rarely, vitiligo.

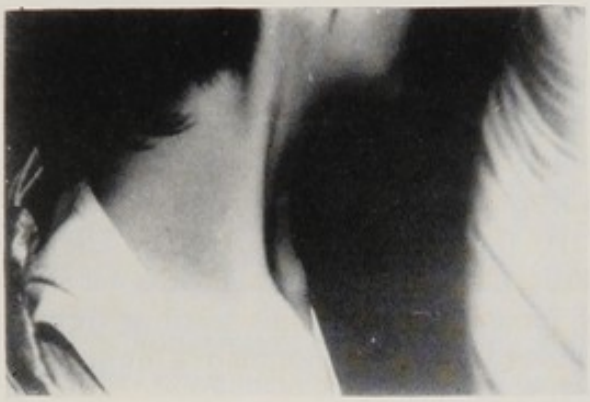


FIG. 9.5. Webbed neck in X0 Turner's syndrome.

(ii) Primary amenorrhoea is always present. The external genital organs keep their infantile features; the secondary sex characters, such as pubic and axillary hair and breasts, do not appear. The uterus and Fallopian tubes are hypoplastic, the gonads absent or reduced, under a thin albuginea, to an ovarian-like stroma without follicular formations. These gonadal rudiments are at the site of the ovaries. In this stroma appear, in more or less large numbers, Leydig-type polygonal cells, often grouped together. In the hilar region these cells may contain Reinke crystals. In this region residues of mesonephrotic canals sometimes appear with elsewhere canaliculi suggestive of undifferentiated testicular tubules.

This dysgenesis is not accompanied by deformation of the external genitals. The clitoris is usually not enlarged; this disorder is associated, after puberty, with hormone disturbances detected by urinanalysis, i.e. reduction in oestrogens with increase in gonadotrophins, an increase sometimes seen in the child (Lindsten, 1963a) confirming a fact noted before chromosomal studies were undertaken (Silver, 1951).

The 17-ketosteroids are normal or reduced, the corticoids rather low, the response to ACTH is normal and thyroid function virtually normal.

Certain cases of X0 are not consistent with this description. Sometimes, there is growth of hair but thinned out, with rudimentary breast development and even within the space of a few years hints of rare menstruations. A bicornuate uterus, and normal Fallopian tubes have been reported. More surprising is the occurrence in an X0 girl of the development of genital organs, breasts, appearance of periods and even, in one case, of pregnancy (Bahner *et al.*, 1960b).

A female of short stature (1·36 m) but with genital organs and breasts of normal shape and regular menstruations at intervals of 5 weeks since the age of 16 years, said that at the age of 31 she had delivered a well-formed boy. The state of the cervix uteri and comparison of the blood groups left no doubt about this birth when karyotypic examination was decided upon. This woman was, in fact, chromatin negative: 24 of 25 cells of skin origin and 51 of 67 from the bone marrow contained only 45 chromosomes. The karyotype analyses demonstrated that this numerical anomaly related to an X0 formula. None of the rare cells with 46 chromosomes (8 of 92) had a normal karyotype. The samplings, one skin, the other two bone marrow, therefore revealed no mosaics. The whole picture made very credible the history of this pregnancy, despite an X0 constitution.

These publications increase the value of observations on chromatin-negative females who, despite this, have menstruated for several years (Hoffenberg *et al.*, 1957, 1959; Greenblatt, 1958; Hutchings, 1959; Bricaire *et al.*, 1961). Among these observations there is one with X0 formula with the usual morphological and hormonal (FSH) signs (J.S.Stewart, 1960b). The others have not been studied cytogenetically. Chromatin, if mentioned, is not always negative. Menses are in the form of spontaneous aperiodic bleeding from the genitals, usually slight discharges followed after a few months or a few years by total amenorrhoea (Boudin *et al.*, 1959).

In fact, these various cases cannot be judged without anatomical examination of the gonads and, unless completely absent, without analysis of their karyotype.

Such cases suggest the hypothesis of an unrecognized XX/X0 mosaicism. This hypothesis must be upheld if cytogenetic checks, which would enable one to discard it, have not been made. These checks, even if they do not reveal mosaicism, do not completely discount this possibility since cytogenetic information is limited to certain cells.

This remark also applies to certain very atypical forms such as observation of an X0 newborn whose appearance was normal apart from oedema of the feet (A.Frøland *et al.*, 1963c).

(iii) Before the adoption of the karyotypic criterion the neuropsychic anomalies in Turner's syndrome had been differently assessed (Doumic, 1950). Certain studies reported frequent mental retardation: 8 in 20 cases reported by Haddad and Wilkins in 1959. Other studies in institutions for mentally backward females (Maclean *et al.*, 1962b) gave in 1962 a proportion of women assumed to be X0 (chromatin negative) of 0·4 per 1000, a frequency equivalent (MacLean, 1964) to that of female newborn chromatin-negative infants (see above).

Many X0 subjects have apparently normal intelligence. However, IQ statistics show a bimodal distribution, the mean of which appears to be a little below that for the general population.

About half the cases are thought to have disturbances in auditory acuity: deafness through disturbed perception more often than disturbance of conduction or of mixed type (Lindsten, 1963a).

The most common form of perceptual disturbance is that revealed in the

audiogram by a deep notch ranging from 500 to 2000 periods/sec and exceeding, between these two frequencies, a loss of 50 db over the normal threshold. This disturbance, apart from the X0 subjects themselves, affects in their families chiefly boys and only their mothers (Wedenberg, 1963). This type resembles certain forms of hereditary deafness, in particular, a recessive X-linked congenital form, but this interpretation cannot be accepted without fuller evidence.

Strabismus, myopia, or hypermetropia, nystagmus, ptosis and blue sclerotics have been reported but not often. The proportion of those with partial colour blindness cannot be exactly estimated but it appears to tend towards a masculine rather than a feminine value.

(iv) The details concerning visceral malformations are no less important. The first observation of Morgagni already referred to a renal anomaly, probably a polycystic kidney. The X0 cases include a reno-ureteral malformation in about one in two, whereas the bladder and urethra appear to be unaffected. From urographic examination these are essentially anomalies in number, structure or position–anomalies in number: double kidney, bifid pyelo-calix, double ureter; anomalies in structure: horse-shoe kidney; anomalies in position: pelvic kidney, malrotation.

These malformations are unilateral or bilateral, isolated or combined.

Cardio-vascular anomalies are much less frequent. They have been noted by various workers (Fraccaro *et al.*, 1960f; Gagnon *et al.*, 1960b; van Gemund and Gelderen, 1961). Apart from coarctation of the aorta, mention must be made of an aberrant subclavicular artery, shrinkage of the portal vein, haemangioma and lymphangioma. Arterial hypertension is sometimes associated with a renal anomaly but not always.

The descriptive value of other malformations is debatable because of their rarity: pyloric stenosis, luxation of the hip. As for the arched palate it is not thought to be particularly frequent in X0 subjects (Lindsten, 1963a).

(v) Another symptomatic feature of X0 subjects is the possible appearance in them with a masculine frequency of X-linked recessive characters. From the point of view of these genes the X0 subjects are "one dose" individuals comparable, despite their sex phenotype, with normal XY males. The animal equivalent of this phenomenon was found in the X0 mouse bearing the characters "scurfy" and "tabby" (Welshons and Russell; W. L. Russell *et al.*, 1959).

The frequency of daltonism would appear to be 8 per cent in these subjects but statistics are still insufficient to verify this proportion. Some workers have drawn attention to the possible interest from this point of view of a variety of Hirschsprung's disease (Hayward and Cameron, 1961b) and of pseudohypoparathyroidism (Schwartz and Walter, 1962).

(d) Clinical evolution

These X0 traits are completed by certain special developmental aspects. X0 subjects are born in general before term and in view of this fact their weight and perhaps even their height are less than average. Oedema of the extremities is noted in about a quarter of the cases. Dental development is sometimes atypical with abnormal persistence of milk teeth and absence of final teeth.

Growth is slow but regular: the osseous age is said to be normal and feminine (Tanner *et al.*, 1959) or a little retarded; the pelvis sometimes remains infantile. This difference between chronological and skeletal age may be accentuated after puberty (Acheson and Zampa, 1961).

After puberty oestrogen therapy helps to establish an artificial menstrual cycle capable of correcting the retardation in osseous age, and of developing hair growth and the breasts to a certain extent, depending on the state of the receptors.

The tendency towards early senility is common (Albright *et al.*, 1942) with osteoporosis of the post-menopause type.

(e) Clinical forms

(i) One of the important consequences of delineation of X0 subjects was assignment of the bilateral Bonnevie–Ullrich X0 status.

After a brief note concerning an infant (Jacobs and Keay, 1959) four cases of Bonnevie–Ullrich status aged from 3 months to 14·5 years (chromatin-negative and X0) were analysed in our laboratory and published in 1961 by Christiaens *et al.*, later followed by another example (Aula *et al.*, 1961). These cases illustrate the transition of the X0 infant with bilateral Bonnevie–Ullrich status to the adult X0 type giving a Turner syndrome with hypergonadotrophinuria. It is worth recording that for a long time in these children paediatricians had known by questioning the mother that neonatal oedema was present on the back of the hands and feet (Sacrez *et al.*, 1958).

(ii) The observations of chromatin-negative monozygotic twins with gonadal dysgenesis (Frasier *et al.*, 1961) have been enriched by two recent examples (H. H. Turner and Zanartu, 1962; Decourt *et al.*, 1964). The first of these sets of twins was monochorionic, the twins were identical and their erythrocytic phenotypes concordant. The gonads of both were reduced to a stroma of the ovarian type without primary follicles. The skin and buccal cells were chromatin negative. These two twin sisters were X0.

The second observation also concerns twin sisters both X0.

(iii) The familial form of Turner's syndrome was described even before cytogenetic studies (Schermann and Renzo, 1950; Klotz *et al.*, 1956). Some of these studies speak of "gonadal dysgenesis" in two sisters (Izakovic, 1960) or even three (Elliott *et al.*, 1959).

Two other cases deserve mention. One concerns two chromatin-negative sisters less than 1·50 m in height, one of them exhibiting a webbed neck (Boehncke and Lenz, 1961). The other case was a small girl and her maternal aunt (Josso *et al.*, 1963) both chromatin-negative. The karyotype of the small girl could be studied; it was X0. That of her mother, a normal female, was XX.

These observations suggest, subject to karyotypic complements, a familial X0 variety. This would mean that this aberration has a feature particularly well illustrated in trisomy 21 and of probable pathogenic interest.

(iv) Gonadal dysgenesis observed in a subject of male phenotype was presented as a variety of Turner's syndrome of probable X0 type (Bloise *et al.*, 1960).

In fact, the striking thing about this 8-year-old boy was the presence of signs of intersex. At the ovarian site on the left was a rudimentary testicle and on the right, elements of ovarian stroma. This gonadal dimorphism gave rise to several interpretations: undetected X0/XY mosaicism or concealed translocation of masculine determinant factors of Y to X or to an autosome.

A similar problem was raised by a coloured subject with phenotype of feminine orientation (Atkins and Engel, 1962b). A testicle without spermatogenesis was found in the left labium major. Laporotomy showed a right Fallopian tube without gonads. The karyotype of this chromatin-negative subject was X0. The possibility of X0/XY mosaicism, however, was not ruled out.

These two observations instead of being considered as a variety of X0 intersex are usually likened because of the anatomo-clinical analogies to the X0/XY type of "true hermaphroditism" (pp. 231 and 232).

2. Apparently normal karyotype varieties in Turner's syndrome

The karyotypic criterion had the great advantage of defining both the characters of the fundamental X0 type and varieties which differ from it in having a normal or pathological gonosomal formula.

(a) XX type

(i) This variety has been the subject of isolated and familial observations. The chromatin body criterion suggested that females with chromatin-positive Turner's syndrome ought to have an XX constitution. Cytogenetics verified this assumption (Decourt *et al.*, 1960b; Fraccaro *et al.*, 1960f; Hauser, 1960; Jacobs *et al.*, 1961c).

A detailed observation of one of these cases (Decourt *et al.*, 1960b) describes a 19-year-old female with primary amenorrhoea and of small stature (1·46 m). Her phenotype was that of a Turner's syndrome. Hypogonadism was primary. Urinary elimination in 24 hr of gonadotrophins (FSH) exceeded 100 mouse units, whereas that of folliculin was less than 5 μg. On the other hand, adrenocortical function appeared normal: 17-ketosteroids 5–9 mg, 17-hydroxysteroids 7·5 mg. The underdeveloped breasts were mainly formed of adipose tissue, the uterus and Fallopian tubes were infantile, the rudimentary gonads formed of connective tissue resembling the ovarian cortex traversed by fibrous bands without Graafian follicles or even a follicular rudiment.

Karyotypic analysis of this chromatin-positive female gave a normal result, XX.

The authors of this publication give two possible explanations to account for the origin of this gonado-somatic dysgenesis. On the one hand, an hereditary influence since the menstrual functions of the mother and those of several female relatives of the father had been abnormal. In addition, a cousin of the father who had menstruated at the age of 16 then suffered from amenorrhoea, then dysmenorrhoea. On the other hand, an acquired process, that is, a destructive lesion of the primary gonads.

Another case, tied up with this because of its XX karyotype and malforma-

tions evocative of Turner's syndrome (webbed neck) (Oikawa and Blizzard, 1961), concerns a female with ovarian tissue verified at laparotomy.

Pterygium colli absent in some subjects with XX Turner's syndrome (Decourt *et al.*, 1960b; Aubert, 1962) may therefore exist in others.

It is useful to compare these single observations, chiefly because of their aetiological interest, with observations on subjects with familial chromatin-positive Turner's syndrome.

The most demonstrative concerns two deaf and dumb sisters, cousins of a deaf and dumb subject and the only children of normal parents. Their maternal grandparents were first cousins. The most obvious symptoms were relative amenorrhoea, excessive elimination of gonadotrophins, and absence of ovaries (coelioscopic control) (Perrault *et al.*, 1951). The first of these patients, obese, with a short neck and cubitus valgus, was 1·41 m in height; the second had a normal appearance; both were chromatin positive. Subsequently, their karyotype was inspected confirming the presumed XX formula.

But most of these familial examples were reported before the use of the karyotype criterion and concern subjects without malformations but with Turner's syndrome and sometimes of normal size, and may fit the diagnosis of "primary amenorrhoea".

Given these reservations, these observations which are distinguished by the high frequency of parental consanguinity (Josso *et al.*, 1963) evoke the idea of recessive autosomal heredity.

(ii) Under this heading we may provisionally place XX "pure gonadal dysgenesis". It was observed (Stadler *et al.*, 1962; Rossier *et al.*, 1961) in three women including two sisters, all XX. An XY form (see below) and another XX/XY (p. 211) have been published.

As for infantile females with hypoplastic "non-functioning ovaries" rich in primary follicles but resistant to gonadotrophins (Klotz, 1958), they are XX from rare examinations including a doubtful one. They may also suffer from anosmia (P. Müller, 1963), thus presenting an "olfactory-genital dysplasia" (de Morsier, 1955, 1963) through unilateral or bilateral agenesis of the olfactory lobes. In one male this state was accompanied by "possible translocation involving a chromosome 16" and by a "voluminous Y". It should be recalled that aplasia of the olfactory lobes (arhinencephalia) is found in half the cases of trisomy 13 (see Chapter 5), and that this aberration may also be accompanied by anomalies of the genital tract.

(b) XY type

(i) Several examples of XY subjects with male phenotype, the appearance of whom by their anomalies is evocative of a Turner's syndrome, justify the view of certain authors singling out a "male Turner" form.

This morbid type being a variety of masculine gonado-somatic dysgenesis is described together with testicular dysgenesis (Chapter 10).

(ii) Another type of chromatin-negative XY but with eunuchoid female phenotype brings together, no doubt provisionally, disparate findings. It can be clearly distinguished from Turner's syndrome.

A case (Hardnen and Stewart, 1959b; J.S.S.Stewart, 1960) provides proof of this: a subject of large stature and female morphology with primary amenorrhoea, undeveloped breasts, high level of urinary gonadotrophins, normal external genitals and uterus of normal size but in whom vaginal exploration did not permit palpation of the ovaries which were not examined. Therefore, by analogy the authors related their observation to cases previously described as "pure gonadal dysgenesis" with absent gonads.

It is not surprising that an XY subject is devoid of testicles if in absence of primary gonads the chromosome Y is not able to induce their development along masculine lines. These cases of "pure gonadal dysgenesis" are, in the view of some, the human replica of early castration (Jost effect): XY or even XX subjects, agonadal with Müllerian development and female phenotype (p. 172).

A genic origin must also be considered. A recent observation is reported with two chromatin-negative sisters, one with pure gonadal dysgenesis and the other with male pseudohermaphroditism (Carr *et al.*, 1961d).

Nor does another observation (Netter *et al.*, 1960; Grouchy *et al.*, 1960) of an XY subject with female phenotype, small uterus and small gonad with Sertoli and Leydig cells in a stroma of ovarian type lend itself to easy interpretation. The presence of a gonad eliminates the diagnosis of pure gonadal dysgenesis and the nature of this gonad a diagnosis of testicular feminization (Miles, 1961); other possibilities, in particular, that of gonosomal mosaicism have been suggested.

(c) Other gonosomal varieties

Many gonosomal aberrations in number or structure have been discovered since their phenotype evocative of a Turner's syndrome attracted attention. Many of these aberrations are examples of mosaicisms (see p. 179).

Type XXX

The first examples of chromosomal aberrations led to the belief that man does not escape the risk of non-disjunction any more than many experimental species. Acceptance of this possibility implies the idea of other varieties of numerical anomalies. Thus, interpretation of the X0 and XXY types suggested as far back as April 1959 the existence of the at that time unknown XXX type (C.E. Ford *et al.*, 1959c).

Study of the chromatin body also justified this research. In fact it brought to light mentally defective females distinguished by the presence of two chromatin bodies in a large number of their nuclei (Barr *et al.*, 1959b) and in the opinion of certain cytologists, two bodies reflected the presence of 3 X (Ohno *et al.*, 1959).

Some months later, Jacobs, Baikie, Court Brown, MacGregor, MacLean and Harnden, 1959c, discovered a chromatin-positive female XXX with two bodies without parallel changes in the "drumsticks". Of average intelligence

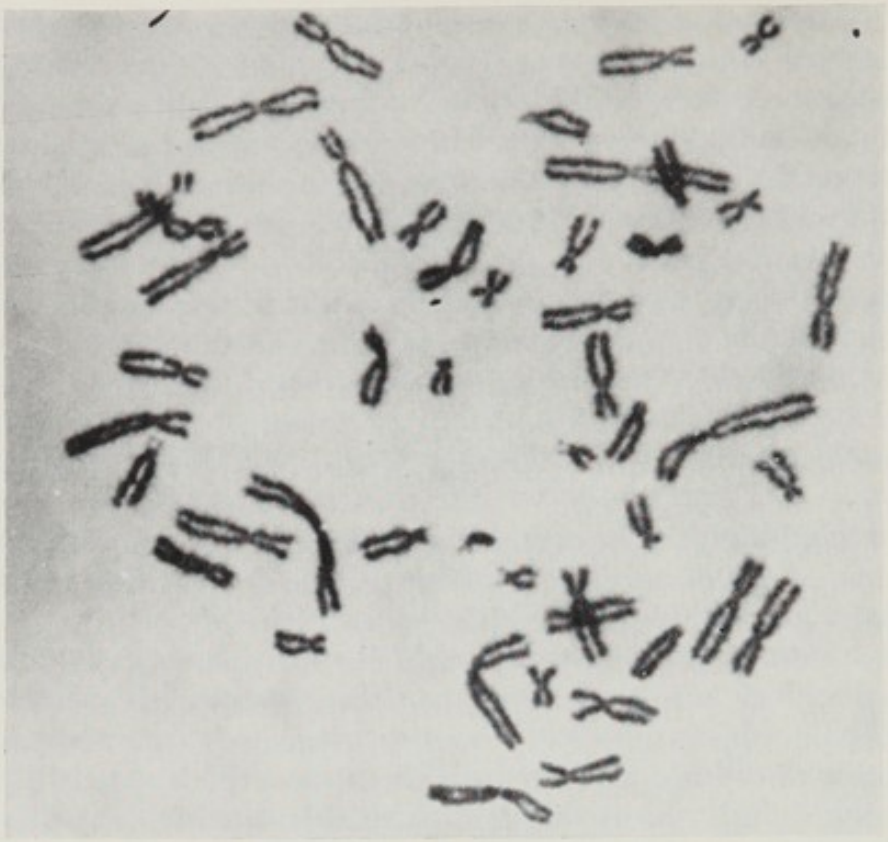


FIG. 9.6. Cell with 47 chromosomes. Triplo X.

she was distinguished by infantile genitalia, late menarche towards 18 years and a functional and early anatomical menopause around 22 years. By analogy with *Drosophila* 3X2A this female was considered as a human example of "super-female". This designation, which Bridges had given to the fruit fly with an extra X, was suggestive of the 3X/2A ratio rather than were its consequences which involve at the same time the sexual regions with hypogonadism and sterility, and nonsexual regions, i.e. abnormal wings and eyes.

In fact, subsequent research was to modify this first clinical impression. Karyotypic analyses of females with double chromatin body detected in an institution for mental deficients led to the discovery of a second case (Jacobs *et al.*, 1960a). But this time the sex characters and the menstruations of this 21-year-old female were normal. The proportions of the cells with one and two chromatin bodies were almost equivalent; 11 drumsticks were found in 500 polynuclears. The XXX karyotype was confirmed by skin and blood examination.

This showed that much could be culled from studies in mental institutions: four cases (blood culture) were detected by the presence of double bodies in 595 mentally defective females (Fraser *et al.*, 1960). These females whose IQ varied from 38 to 58 were epileptics, all belonging to the A group. The antecedents of the eldest, 73 years old, could not be traced but the three others had normal or almost normal cycles.

These inquiries drew attention not only to the mental state of these XXX sub-

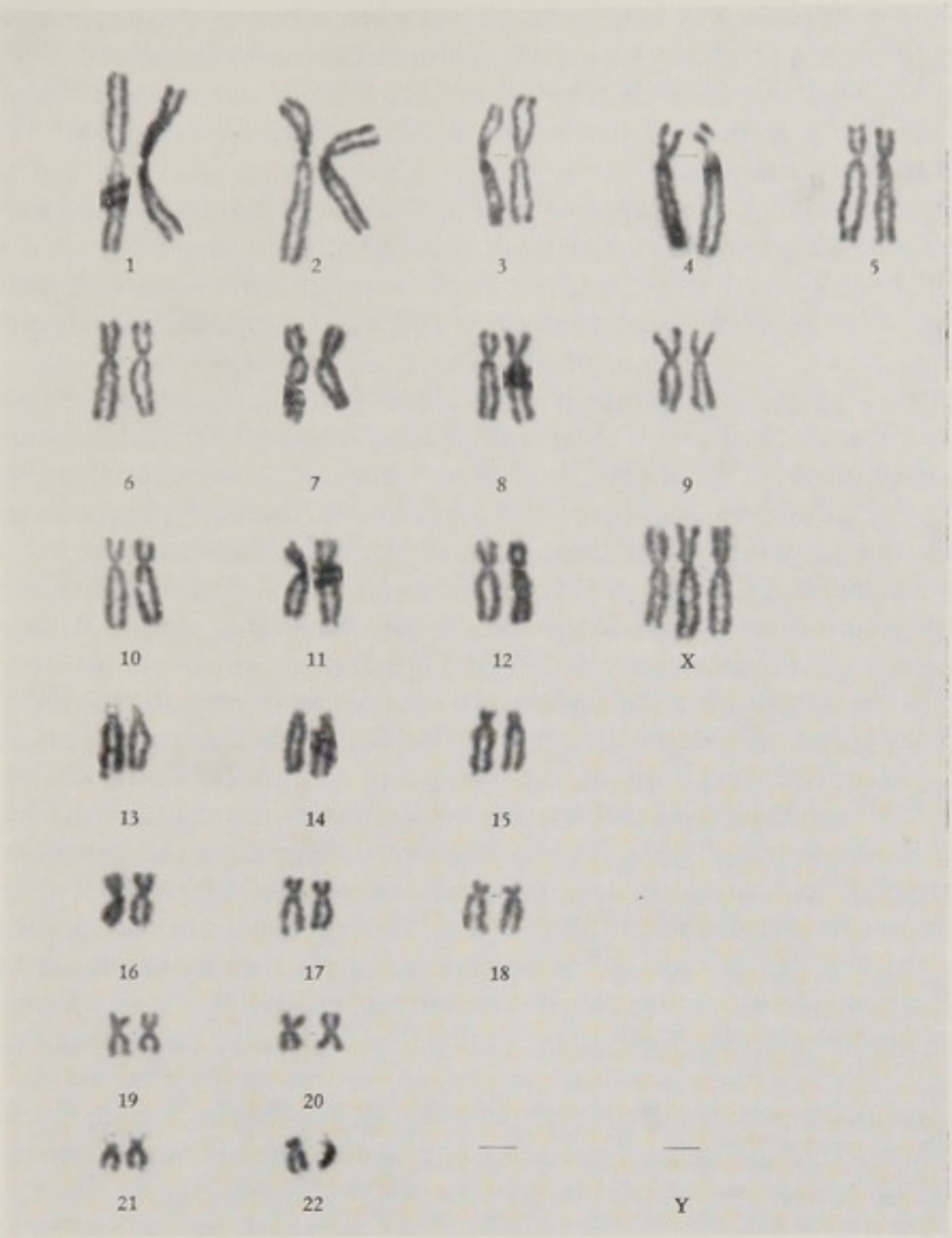


FIG. 9.7. Karyotype with 47 chromosomes. Triplo X.

jects but, contrary to the first impression, to their ovarian activity and even fertility. One of these four women had, in fact, a normal son, chromatin-negative and of XY karyotype.

These initial findings supplemented by later observations helped to sort out ideas on the frequency, conditions for appearance and physical and psychic characters of this new morbid type. The characters of the drumsticks were also defined (p. 282).

(a) The frequency of the XXX type was estimated by MacLean (1964) during the inquiry mentioned (p. 164) at 1·2 per 1000 live births (12 per 10,000).

This type of gonosomal aberration is therefore not exceptional. The double

chromatin criterion may help to identify it when a karyotype interpretation is made difficult by similarity between chromosomes X and 6 (Sandberg, 1960a) or by possible deletion of an X (De Carli *et al.*, 1960; Jacobs *et al.*, 1960b). The size and DNA content of the bodies in XXX subjects are normal (Lindsten, 1963a).

Some attempts at identification by the method of ^{3}H-thymidine have also given interesting results (Mukherjee *et al.*, 1963b; Giannelli, 1963) (p. 281).

(b) The risk of a birth of a triplo X girl appears to increase with maternal age but more evidence is necessary to establish this hazard (Johnston *et al.*, 1961c).

Further, following a law which seems almost general, the triplo X may coincide in the same family with other chromosomal aberrations. Various examples have been given: XXX/XXXX mother and trisomy 21 daughter (Bergemann, 1962); XXX daughter and trisomic 21 brother; XXX daughter and trisomic 21 cousin, the daughter of a paternal uncle (Breg *et al.*, 1962a).

Karyotypic study of the mother, father and sister of a triplo X female gave normal results (Stewart and Sanderson, 1960d). Such study, despite its interest, does not appear to have been undertaken often.

(c) In the same subject the coincidence of triploidy X with an autosomal trisomy has been reported: with trisomy 18 (Uchida and Bowman, 1961a; Uchida *et al.*, 1962b; Ricci and Borgatti, 1963) and with trisomy 21 (Day *et al.*, 1963b).

(d) Although there is no characteristic malformation in subjects with triplo X, and although some of these females may not diverge from the norm in their menstrual functions and in their physical and psychic state (Close, 1963), it should not be deduced from this that they always have a normal appearance. Since the first publications, some observations have mentioned physical anomalies: mongoloid slanting palpebral fissure (de Carli *et al.*, 1960); webbed neck (Sandberg *et al.*, 1960a); congenital hydrocephaly; and obesity (Barr, 1962d).

This said, the most striking signs are fertility in presence of mental deficiency.

(e) The fertility of these women in spite of the extra X is now proven by several observations.

1. A mentally defective female born in 1925 giving birth to four boys (Stewart and Sanderson, 1960d). These children at the time of examination were 12, 10 (two dizygotic twins) and 8 years old. Mental and physical development were normal, chromatin negative and their chromosome formula had not been established. The report did not mention any miscarriage but a fifth pregnancy then in the third month.

2. A mentally defective and epileptic female, mother to a normal 3-year-old boy at the time of examination, who was chromatin negative with a normal XY karyotype (blood culture) (Fraser *et al.*, 1960).

3. A 61-year-old mentally defective and schizophrenic female (IQ = 50) with nine pregnancies (Barr, 1962d): five boys and four girls. Two of the boys succumbed at an early age and a third was killed during the 1939–1945 war. The chromatin type of five of the survivors (two mates and three females) could be obtained; it was normal in relation to clinical sex.

4. A 63-year-old mentally defective female (IQ = 43) with a 20-year-old illegitimate daughter whose chromatin could not be verified (Barr, 1962d).

5. A practically normal female without obstetric incidents, the mother of two normal chromatin-negative XY boys (Close, 1963).

That is, in all, 12 boys and five girls with absence of information on two boys who died at an early age, with three of five girls verified as chromatin positive. For 8 boys mention is made of normal somatic and psychic development; for the other children, no mention is made of an abnormal state. In the absence of pathological histories, apart from the two boys who died at an early age, it therefore seems probable that the progeny known to date of triplo X women are not marred by serious stigmata except for the case of the female trisomic 21 born to an XXX/XXXY mother (see p. 176).

And yet the XXX female may, in principle, produce XX and X ovules in equivalent numbers. The fertility of the XX ovule and the viability of the XXX zygote are demonstrated by the very existence of XXX females.

While a trisomic 21 female may yield diplo 21 ovules it seems at present that the triplo X female cannot produce diplo X ovules. However, the material studied is still too sparse to warrant a conclusion. Time may reveal in the progeny of triplo X females the presence of XXX and XXY children, and triplo X females may also possibly be detected among mothers of XXX and XXY subjects.

The same considerations apply to XXXX, XXXY or X0 subjects.

(f) Mental deficiency actually appears to be the most constant abnormal consequence of the extra X. Since the first communication, new observations also mention mental deficiency (Johnston *et al.*, 1961c) and sometimes also various mental disorders (Kidd, 1963).

Some have rightly asked whether the way the inquiries were run did not lead to selection of institutionalized mental defectives missing females whose intelligence is compatible with normal life. Combining the results of the various inquiries (Fraser *et al.*, 1960; MacLean *et al.*, 1962b; Johnston *et al.*, 1961c) based on the chromatin criterion and carried out in the same way in centres for the mentally backward, the proportion of the XXX type is 4·51 per 1000 (15 per 3329). Comparison of this result with that in the inquiry among newborn girls at 1·2 per 1000, testifies to a significantly higher frequency of females of XXX constitution in the group of mentally backward, assuming that the mortality within each of these two groups is the same. However, the IQ of XXX subjects is said to be over 20 (Breg *et al.*, 1963).

XXXX type

The first two examples of females of this chromosomal type were detected by the chromatin criterion in an institution for mental defectives by Carr, Barr and Plunkett (1961d). They are distinguished by the presence among nuclei with one or two chromatin bodies of others with three bodies.

The first, aged 13 years, did not deviate from the physical norm for her age:

height 1·56 m, satisfactory development of sex characters, menarche at 12 years 3 months and, since then, normal periods. Examination of the endocrinal system, apart from indications of slight disturbances in thyroid activity, showed no anomalies, as was also true of urinary hormonal excretion. On the other hand, her non-psychotic mental deficiency was severe: mental age of about 4 years with an IQ of 30.

Study of jugal chromatin revealed in 100 cells 17 with one, 35 with two and 43 with three sex chromatin masses and equivalent proportions in the vaginal cells, whereas in the skin cells, the proportion of cells with three masses was less and those of cells with two higher. In 1000 polynuclear neutrophils 42 drumsticks were counted.

Chromosome study of 110 blood cells revealed 102 with 48 chromosomes and karyotype analysis of 21 cells was conclusive and showed very probably an XXXX formula. The practically normal parents of this child were not consanguineous. Two maternal aunts were mental defectives.

The second patient aged 32 was very similar to the previous one with satisfactory physical development, height 1·61 m and with normal menstruation. Apart from moderate and diffuse swelling of the right thyroid lobe no physical anomaly was apparent. Non-psychotic mental deficiency for which she was admitted corresponded to an IQ of about 50.

Analysis of the chromatin also revealed along with cells with one or two, cells with three masses. The skin, as with the preceding case, showed more cells with two and fewer with three than did the jugal cells.

Analysis revealed in 124 cells 99 with 48 and 15 with 47 chromosomes, the latter being too dissimilar to justify hypothesis of a mosaic. As for the 48 chromosome karyotype, it most probably corresponded to a gonosomal XXXX formula.

Verification of the chromatin body in the non-consanguineous parents of both patients, their mothers in particular, and their relatives, could not be made.

In their comments the authors consider another explanation for the presence of cells with three bodies, that of tetraploidy, but cells with three bodies were found in the mucosal and skin regions whereas polyploid cells when they are seen are encountered in a few rare territories—liver (Reitalu, 1957; Ohno *et al.*, 1959), amnion and bronchial epithelium. The discovery by culture of leukocytes of tetraplo X cells suggests that the cells with three chromatin masses in the mucosal and skin regions must also be tetraplo X.

An XXXX female with a trisomic 21 daughter (Bergemann, 1962) provides a further example of familial association of chromosome aberrations.

XXXXX type

Because of overall retardation of development, a patent ductus arteriosus and some minor anomalies of the facies and extremities, the karyotype of a 2-year-old girl was obtained and revealed the presence of three extra chromosomes in the 6–12 X group. Since a ^{3}H-thymidine test had shown the asynchronous

synthesis of four chromosomes of this group, the authors considered that these were X supernumeraries (Kesarée, Wooley and Samson, 1963). Chromatin study of the buccal cells revealed nuclei with four bodies. This abnormal girl, without deformed external genitals, may therefore be considered as a 44 A XXXXX type. Analysis of the "drumsticks" did not give results parallel with those obtained from analysis of the chromatin masses but some nuclei with two and even three appendages were found.

The state of this infant permitted successful ligation of the ductus arteriosus.

This observation shows that the pentaplo X organism envisaged by theory may be viable and even capable of withstanding considerable surgical trauma.

Observations on the chromosome types X0, XXX and XXXX provide valuable information on the consequences of being minus one X or having one or two extra X chromosomes. Total or even partial absence of an X gravely compromises ovarian development, presence of one of two extra X chromosomes does not, mainly impeding psychic awakening. The symptoms of triploidy and tetraploidy X are, in fact, according to observations so far reported, very similar.

These findings are amenable to interpretations developed elsewhere (pp. 284 and 285).

II. Forms with mosaicism

The same individual may have several types of cell distinguished by different karyotypes. These chromosomal mosaics involve isolated or associated numerical or structural aberrations.

The gonosomal varieties were the first to be described. They were even foreshadowed in two cases of Turner's syndrome when nuclear chromatin types differing according to the skin regions examined were detected (Danon and Sachs, 1957). A possible X0/XX mosaic was then suggested.

The karyotype reality of the phenomenon was established by Ford, Polani, Briggs and Bishop (1959g) when in checking on the XXY constitution in Klinefelter's syndrome they discovered in a patient the possibility of an XX/XXY mosaic (bone marrow). Since then, the diversity of gonosomal mosaics has steadily increased. It is so far broader than that of the autosomal varieties. Certain forms have been described involving hermaphroditism and pseudohermaphroditism (see p. 230 et seq.).

Whether gonosomal or autosomal mosaics they cannot be considered without some reserve due to the difficulty of their study. At first, diagnosis of chromosomal mosaicism must be based on criteria eliminating artefacts. The proportion of atypical cells must be higher than that which may be reasonably expected by chance. The diagnosis is more probable if this surplus is found in a large number of preparations of the same tissue and from different tissues.

However, two or more clones may be mingled in the blood or bone marrow and occupy distinct skin or aponeurotic areas. Moreover, mosaicism may be confined to the bone marrow or the blood and not extend to other tissues.

Again, its limits in space are impossible to define since our techniques for

accessible regions only cover too limited sectors and leave aside virtually inaccessible regions.

Its evolution in time is completely unknown. And yet it is quite possible that selective processes modify over the years the picture of the mosaic responsible in the initial period of embyrogenesis for dysgenesis definitive from the outset.

(1) X0/XX variety

Identified early on and often found (Ford, 1960b; Fraccaro *et al.*, 1960c; Sandberg *et al.*, 1960b; Ferrier *et al.*, 1961, 1962; Jacobs *et al.*, 1961c; Grouchy *et al.*, 1961c, 1962a; Bompiani *et al.*, 1961; Lindsten, 1963a; Haddad, Jr., 1962; de la Chapelle, 1962a), this mosaic gives a Turner's syndrome of which the similarities to and the differences from the X0 type are worth investigating.

The similarities are the small stature, practically always primary amenorrhoea, android morphology, shortness of the neck, spacing of the nipples, cubitus valgus, very often high level of the gonadotrophins and gonadal aplasia and uterine hypoplasia. A deformed kidney (horseshoe kidney) sometimes completes this analogy.

The differences are found in the following characters more peculiar to X0: the even shorter stature, webbed neck and epicanthus, possible retardation of mental development with an IQ a little less than 100 on average and bimodal distribution, nearly always with absence of development of the secondary sex characters. There may be coarctation of the aorta and oedema of the extremities at birth.

It is therefore not impossible that the consequences of an X0 karyotype may be attenuated, depending on the site and size of the XX clone. The proportion of the two clones may also vary with time.

The chromatin body sought in buccal cells or culture has been found in nearly half the observations published.

In several observations with mosaic observed in the leukocytes and with XX cells in the skin, buccal chromatin was positive. In other cases of mosaic observed in the bone marrow and with X0 cells in the skin, buccal chromatin was negative. These facts point to a relation between the chromosomal type of the skin cells and chromatin type of the buccal cells.

(2) X0/XXX variety

This constitution was at first attributed (Jacobs *et al.*, 1960a) to a female of small stature whose outstanding features were primary amenorrhoea, hypoplasia of the external genitals, clitoris excepted, absence of vagina and with masculine proportions of the shoulders and pelvis. The uterus was not palpable, chromatin was positive with 52 per cent cells with one chromatin mass and 37 with two. No drumsticks were found in 1500 polynuclears. The bone marrow revealed an X0 formula in nearly all the cells while blood and skin had an X0/XXX formula.

Another publication (Jacobs *et al.*, 1961c) concerns a female whose genitals were simply hypoplastic and the gonads reduced to rudimentary elements. The jugal chromatin was positive with a majority of cells with one chromatin mass although others had two.

Examination of the blood revealed 88 per cent of cells with 45 chromosomes mainly X0 and 5 per cent with 47, XXX. Examination of the skin revealed a majority of X0 cells with XXX cells more numerous than in the blood cells. In addition, in these two tissues only about 2 per cent XX cells were counted.

The authors concluded that these two cases probably had an X0/XXX mosaic not ruling out the possibility of an X0/XX/XXX variety which might have been seen on examination of tissues not, in fact, studied.

On the occasion of an acute myeloblastic leukaemia which led to her death, a 64-year-old female, mother of three normal sons, was the subject of a karyotype study (blood and bone marrow) (Lewis *et al.*, 1963e) revealing in 138 cells, 88 with 45, three with 46 and 45 with 47 chromosomes. The cells with 45 chromosomes were X0, those with 47, XXX. Of 100 buccal cells 28 had no chromatin mass, 35, one, 34, two and 3 three masses. The authors made the diagnosis of an X0/XXX mosaicism. But it cannot be excluded because of the percentage of buccal cells with one mass that a skin culture would have revealed a proportion of XX cells with 46 chromosomes higher than in the blood and bone marrow. This observation is a further example of leukaemic complication of a chromosome aberration. An X0/XXX mosaic implies non-disjunction during the first cleavage of the zygote; later, the non-disjunction may produce an X0/XX/XXX mosaic.

These mosaics, just like any numerical gonosomal anomaly, justify identification of the X chromosomes with marker genes. In this connection the observation (Thuline *et al.*, 1962) of an X0/XXX girl with no disturbance in colour perception, a disturbance from which her father suffered, is worthy of note.

(3) XX/XXX variety

The female who furnished this example of mosaic (Grouchy *et al.*, 1961e) was not distinguishable at first sight from a normal female but she had only menstruated once at the age of 21 years.

The urinary gonadotrophins eliminated in 24 hr varied from 5 to 25 mouse units but the ovaries were large, smooth and hard and their histological features compatible with diagnosis of Stein-Leventhal syndrome. A cuneiform resection of the ovaries was followed 30 days later by menstruation and start of normal cycles.

The jugal chromatin was positive: 30 per cent of the cells with one, 2–3 per cent with two masses. Karyotypic analysis (fibroblast culture) indicated an XX/XXX mosaic. According to the authors of this publication, several forms of Stein-Leventhal syndrome depend on chromosomal anomalies, i.e. this usual form and two other forms (pp. 222 and 216) by deletion of the long arm of one of the X chromosomes, one being minor in the mosaic form, the other serious without mosaic. Another form reported is X0/XX/XY (Netter *et al.*, 1961).

These results were not confirmed by subsequent studies. One communication in particular, mentions an observation of Stein-Leventhal syndrome with normal karyotype (skin and blood) and ten cases of polycystic ovarian sclerosis with normal karyotypes (blood and ovary) (van Campenhout *et al.*, 1963).

Cytogenetic analysis by blood culture supplemented twice by ovarian biopsy culture revealed no anomalies in these ten sterile women with polycystic ovarian sclerosis, oligomenorrhoea, secondary amenorrhoea and sometimes, obesity, hirsutism, or virilism. In addition, the ovarian cells studied contained one sex chromatin mass.

The observation of a young woman (Jacobs *et al.*, 1961c) of normal body form with primary amenorrhoea at the age of 19 years, rudimentary Fallopian tubes and ovarian tissue without follicles warrants discussion. While the formula of the blood cells was XX, that for the skin and peritoneum had 46 and 47 chromosomes, the cells with 46 were of the XX type and those with 47 possessed an extra medium-sized chromosome. But since the jugal and skin cells contained only a single chromatin mass the cells with 47 chromosomes were not considered XXX but XX with a trisomy 10 or 11.

The formula XX/XXX of a mentally defective female (MacLean *et al.*, 1962b) shows that psychic anomalies may bring XX/XXX close to XXX.

At any rate, since the XX/XXX formula may result from loss of an X during the first cleavage of an abnormal XXX zygote, the maternal chromosome formula may provide a useful indication.

(4) X0/XX/XXX variety

Various examples of this type of mosaicism would have gone unnoticed if chromosome diagnosis had not been based on repeated samplings of skin and blood or blood and the bone marrow.

The first observation (Grumbach *et al.*, 1960, 1961) of a 13-year-old girl was at first interpreted as a dysgenesis of X0 type with positive chromatin. This unusual antagonism was based, on the one hand, on the presence of over 60 per cent buccal, vaginal and skin cells with one chromatin body. Over 6 per cent of the neutrophil leukocytes had nuclear appendages. The X0/XX/XXX mosaic was discovered at the same time as the cells with two chromatin masses by examination of a third skin biopsy. This mosaicism was verified by blood culture.

The clinical interpretation of another observation (Hayward and Cameron, 1961b) is difficult, since the girl in question, stunted and mentally backward, had suffered at 11 months from tubercular meningitis. Supra- and ante-sellar calcifications recalled this grave incident at an early age. At 7 years her IQ was 64; at 10 years her height was about 1 m but the external genital organs and urinary hormone elimination corresponded to the pre-puberty stage, still without any development of the breasts or hair growth. Some common slight dystrophies (wide inter-nipple distance, fusion of toes) completed this set of symptoms which did not include the results of direct examination of the ovaries.

While the bone marrow karyotype established by sampling was X0, that of the blood was X0/XX/XXX with weakly positive buccal chromatin but with cells with one or two chromatin masses.

The same author reports the coincidence of X0/XX/XXX mosaicism, single sex chromatin mass and Hirschspung's disease.

Another observation (Carr *et al.*, 1962c) confirms the need to look for chromatin and chromosomal types in different regions. A female born in 1934 had

from her clinical and hormonal features been considered as suffering from a variety of Turner's syndrome without mental retardation or psychic disturbance, but buccal chromatin analysis revealed 36 per cent with one and 12 per cent with two, vaginal analysis 53 per cent with one and 19 per cent with two chromatin masses. This result did not accord with the blood X0/XXX karyotype. The skin karyotype, on the other hand, appeared concordant X0/XX/XXX.

As for the percentage of chromatin appendages in the polynuclear leukocytes it was less than usual, as already noted in one X0/XXX case (Jacobs *et al.*, 1960a).

The ^{3}H-thymidine method completed the cytogenetic analysis of a case reported by Morishima *et al.* (1962).

The X0/XX/XXX mosaicism may arise from various disturbances in normal segregation of the X chromosome. The simplest is non-disjunction during division of one of the first blastomeres giving two daughter cells one XXX and the other X0 which will develop jointly with the normal XX line derived from the other blastomere. This explanation was proposed by Stern (1960b) because of its simplicity, recalling that he had found in 1933 in *D. melanogaster* an example of this mosaic, human pathology now furnishing a replica.

(5) X0/XYY and X0/XY varieties

Because of their female phenotype two subjects of short stature, with primary amenorrhoea, chromatin-negative and X0/XYY deserve to be mentioned here.

They are described together with examples of gonosomal mosaicism involving the Y chromosome (pp. 212 and 213) and X0/XY cases evocative of Turner's syndrome (p. 210).

(6) XXX/XXXX variety

This variety was found in a female, one of her daughters and granddaughter. The sister of the latter was XX but with trisomy 21. In addition, the maternal aunt of these two children was XX/XXX/XXXX (Bergemann, 1962).

CHAPTER 10

Numerical Gonosomal Aberrations

TESTICULAR DYSGENESIS

I. Forms without mosaicism

Non-disjunction of the X chromosomes at anaphase II in oogenesis results in an XX daughter cell and the other devoid of an X chromosome. Fertilization of the latter by a Y spermatozoid should give an 0Y zygote which has never been identified. It is probable that this zygote is not viable which would be in man the replica of a fact established in *Drosophila*. In fact, an ovule, without an X chromosome is fertile since a subject with Turner's syndrome may draw the X from his father (p. 266).

In 1942, Klinefelter, Reifenstein, Jr. and Albright described in man a syndrome involving a gynaecomastia, increase in urinary gonadotrophins, hypotrophic testicles with aspermatogenesis, tubular fibrosis, hyalinization and presence of Leydig cells.

(a) This communication, based on nine cases, brought together the essential anatomo-clinical and hormonal features of this syndrome now generally known as Klinefelter's syndrome. Subsequent descriptions confirmed and more clearly defined the previously noted features.

A notable aspect of the nine subjects studied aged from 17 to 38 years was the contrast between development of secondary sex characters and the consequences of testicular dysgenesis.

On the one hand, the penis, the prostate and hair cover were normal; on the other, the testicles were very small with azoospermia. Postpubertal gynaecomastia developed.

In seven of these patients histological examination was made. With different shades of intensity the same essentially tubular lesions were recorded: atrophy and hyalinization of the seminiferous tubules, absence of spermatogenesis and inflammatory signs, in presence of abundant and even hyperplastic interstitial cells.

These histological signs concurred with the increased urinary elimination of gonadotrophins as is seen in castrated subjects while elimination of 17-ketosteroids was normal or subnormal.

This special type of masculine gonadal dysgenesis thus appears to affect selectively the tubular functions and pass over Leydig functions. The authors assume that excess of FSH was due to deficiency of a tubular hormone (inhibin).

Klinefelter's syndrome is not an example of intersexuality. The small and

hard testicles have migrated normally into the sacs, development of the breasts gives gynaecomastia of variable size, a common consequence of testicular hypoplasia and not a breast of feminine morphology as in the hermaphrodite; hypospadias is usually not reported and folliculinuria in most cases is normal. Androgen excretion is not sensitive to the stimulatory effect of chorionic gonadotrophin, testicular androgens are secreted in very low amount and may be present in abnormal form, while suprarenal inhibition by dexamethasone sometimes stops elimination of 17-ketosteroids.

Despite this hypoplasia with sterility, the XXY subject is in many cases sexually active and possible fecundity here has even been discussed (see p. 193)

(b) The chromatin body criterion was applied to study of Klinefelter's syndrome as far back as 1956 (Bradbury *et al.*, 1956; Plunket and Barr, 1956; Nelson, 1956).

A large number of these patients, 50–80 per cent, then appeared to be chromatin positive.

This sex chromatin study enabled Ferguson-Smith *et al.* to detect in 1957 among 91 azoospermic or oligospermic subjects, 10 chromatin-positive subjects. Just as the chromatin criterion by itself led to the idea that Turner's syndrome had a chromatin-negative XY formula, an example of sex inversion in the female, here it led to the assumption that these males had an XX formula.

(c) The first attempt at chromosomal analysis favoured this hypothesis. In fact, Ford *et al.* (1958d) examining by bone marrow sampling a subject with chromatin-positive Klinefelter's syndrome found in five "good" cells at metaphase, four with normal female XX karyotype and the fifth without doubt an XX cell. This observation appeared to confirm the presumed reversal of the genetic sex; subsequent investigations were not to confirm it.

In a note of 31 January 1959 Jacobs and Strong (1959f) announced that they had observed in a subject with Klinefelter's syndrome a great majority of cells (bone marrow) with 47 chromosomes, compatible with "possible XXY sex determination".

This figure of 47 was found again in the following April by Ford *et al.* (1959g) in bone marrow samples at the same time as other cells with 46 chromosomes. These authors suggested the possibility of an XX/XXY mosaic, the bone marrow alone being considered. This formula was again reported in other subjects who were also assumed to have Klinefelter's syndrome.

Since then, comprehensive clinical and anatomical observations of a hermaphrodite have shown (Turpin *et al.*, 1962) that gonosomal XX/XXY ambosexuality may be accompanied by phenotypic ambosexuality (cf. hermaphroditism).

Nosological consequences

The karyotypic criterion now distinguishes various morbid types previously grouped under the common name Klinefelter's syndrome. A well-defined anatomo-clinical type does not always correspond to each of these karyotypic varieties. The best known is the XXY variety.

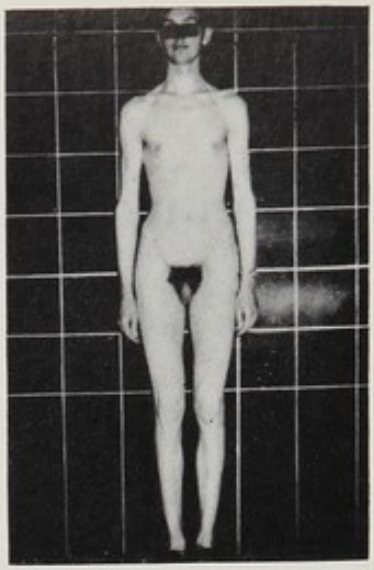


FIG. 10.1. Subject with XXY Klinefelter's syndrome.

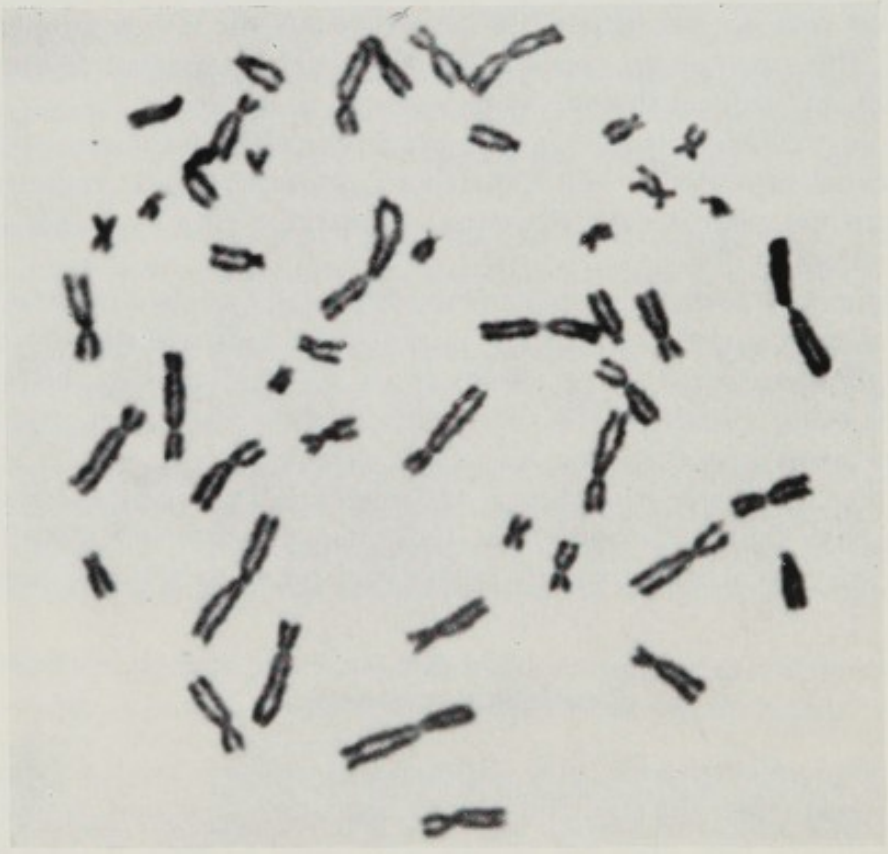


FIG. 10.2. Cell with 47 chromosomes. XXY Klinefelter's syndrome.

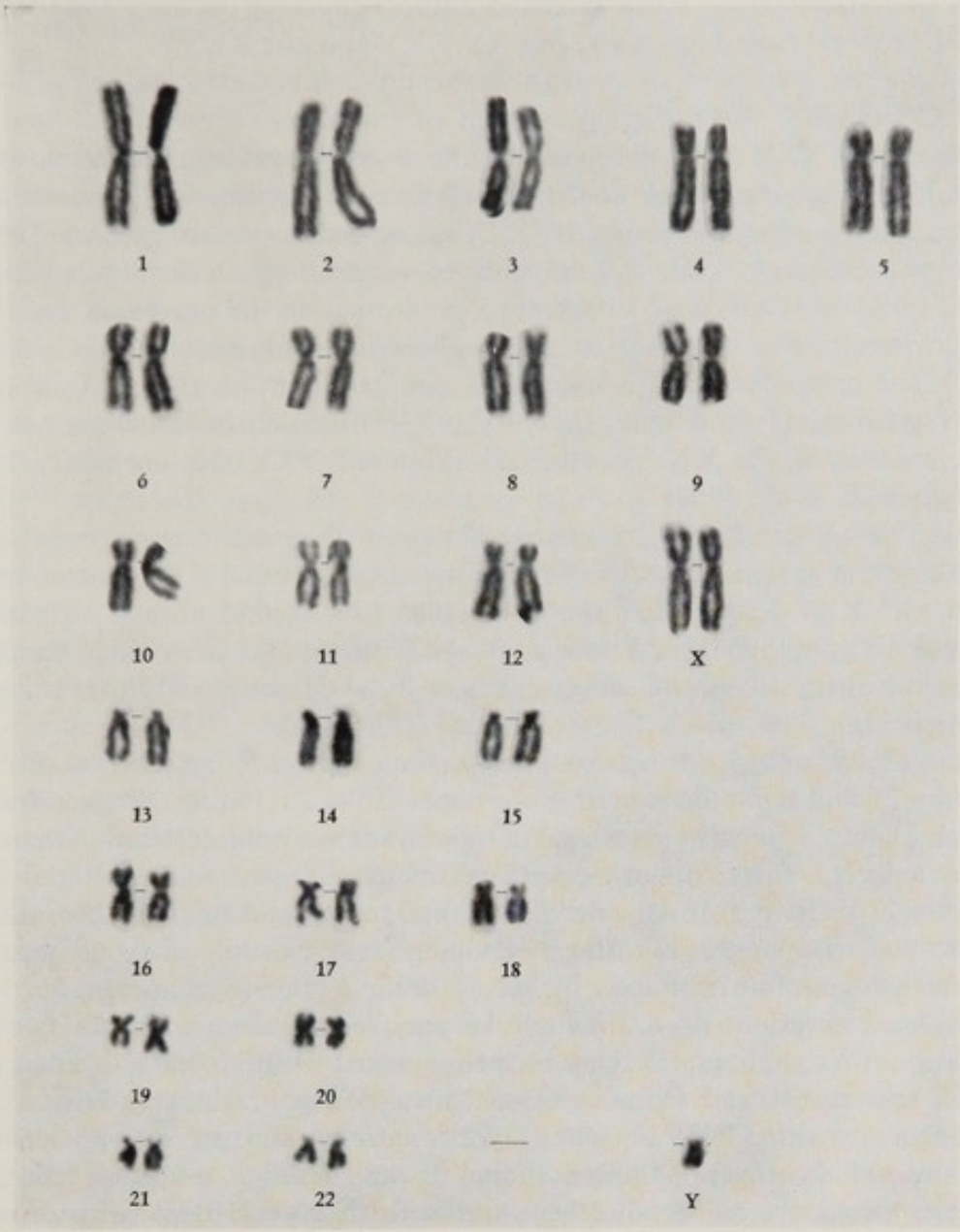


FIG. 10.3. Karyotype with 47 chromosomes. XXY Klinefelter's syndrome.

I. XXY type

This cell type is characterized by the presence of an extra chromosome linked by morphology to group 6–12 X and by the presence of a chromatin body at interphase.

(a) Frequency

The results of inquiries in various countries on the proportion of chromatin-positive live newborn boys are more or less the same: 5 of 1911 (Moore, 1959); 4 of 1890 (Bergemann, 1961b); 21 of 10,725 (Court Brown *et al.*, 1964), i.e.

30 of 14,526 or 2·06 per 1000. The latter authors checking the karyotype of 16 of the 21 neonates found ten XXY, five XY/XXY and one XXYY.

(b) Conditions for appearance

Presence of XXY seems to be greatly influenced by certain familial conditions.

(i) The frequency of this aberration appears to increase with maternal age (Lenz, 1959b, Part II; Penrose, 1959; Ferguson-Smith, 1960b; Stewart, 1960c).

There is reason to relate this influence of maternal age to the familial coincidence quite often noted of Klinefelter's syndrome, on the one hand, and twinning, trisomy 21 or other chromosomal aberrations, on the other.

(ii) The coincidence of twinning has claimed attention (Hoeffnagel, 1962) whether involving the family, sibs or the XXY subject. In the latter case the twin brother of the XXY is normal or himself XXY (Holub *et al.*, 1958; Nowakowski *et al.*, 1959).

(iii) The coincidence of trisomy 21 has been observed in the progeny of a female with a normal karyotype who, with her first normal XY husband, had a child with XXY Klinefelter's syndrome, then to a second normal husband, a normal XY son, then from a third normal XY husband, a trisomic 21 daughter apparently with 46 chromosomes due to a 21 ~ G translocation (Bernischke *et al.*, 1962e).

(iv) Familial coincidence at one and the same time of Klinefelter's syndrome, trisomy 21 and twinning is even more remarkable. An inquiry directed by the search for sex chromatin mass among the boys of a mental institution detected in ten subjects with chromatin-positive Klinefelter's syndrome, two related to trisomy 21 subjects (Mosier *et al.*, 1960a). One had two twin brothers one of whom was trisomic 21; the other, surviving from a couple of twins, was the uterine brother of a trisomic 21 by his mother in a previous marriage.

These observations do not include karyotypic analyses. Since then two observations (Wright *et al.*, 1963) have been reported with familial coincidence of XXY, trisomy 21 and twinning. One concerns five brothers and sisters including a boy with XXY Klinefelter's syndrome, a normal girl and a normal boy and two twin brothers one of whom had trisomy 21.

The other concerns four brothers, one with trisomy 21, two twins of male sex including a stillborn infant, and a boy with chromatin-positive Klinefelter's syndrome (five XY and five XXY cells).

Finally, this coincidence has been noted in monozygotes one XXY, the other trisomic 21 (Hustinx *et al.*, Turpin *et al.*) (pp. 77 and 193).

The coincidence of XXY with trisomy 21 is a further example of familial encounter of chromosomal aberrations. In one of our cases this aberration was no longer numerical but structural: balanced paternal 22 ~ D translocation transmitted to the son who in addition had an XXY formula (Institut de Progénèse, No. 293).

Concerning these facts it is useful to record that the kin of XXY subjects is not infrequently distinguished by a varied pathology: endocrinal, for example, athyreosis; neuro-sensory form e.g. myotonic dystrophy, cerebellar ataxia, pigmental retinitis; and the mental form, schizophrenia for example.

(c) Semeiology

(i) The anatomo-clinical and hormonal features of most XXY subjects are in line with the previous description of Klinefelter's syndrome. They permit diagnosis, once puberty has passed.

Tallness is common with macroskelia. Hair on limbs, trunk and face is scanty. Very frequently more or less considerable gynaecomastia develops at the time of puberty. Often it does not exceed the size of a hazel nut or a walnut seen under the areola.

These features sometimes give the subject a feminoid appearance or the eunuchoid aspect considered below.

The essential element is primary hypogonadism frequently associated with mental deficiency and even psychopathic disturbances.

(ii) Primary hypogonadism assumes all its traits in the adult. The penis is small or of normal size, testicles are always small but of firm consistency and of average length less than 1·5 cm. Unilateral or bilateral cryptorchidism is reported.

This hypogonadism is described as primary as it is accompanied in all cases by abnormally high urinary elimination of gonadotrophins generally above 100 mouse units (Decourt, 1960a). The levels of 17-ketosteroids are in general a little down, those of the 17-hydroxysteroids normal; it is rare for folliculin to be increased.

The histological features of the adult XXY testis also concur with the classical description. Most of the seminiferous tubules are atrophied and completely hyalinized, some wider ones contain Sertoli cells. The interstitial tissue forms clumps sometimes arranged in pseudo-adenomatous strip zones. The Leydig cells are usually dystrophic, poor in lipids, devoid of Reinke crystals and eosinophils, and sometimes they are monstrous with hypertrophy of the nucleus and nucleolus. Sclerosis is variable without inflammatory signs.

This hypogonadism, together with the signs already mentioned, very scanty hair and gynaecomastia is accompanied by skeletal peculiarities such as excessive length of the lower limbs with girth greater than the length, while minor deformations of the fingers and toes complete the morphology peculiar to these patients. Not so marked are kyphoscoliosis, internal frontal hyperostosis, telangiectasis and angiomatosis.

(iii) The discovery of the karyotypic criterion was followed by inquiries into the psychic features of this XXY variety of Klinefelter's syndrome.

These patients are often distinguished by their passive–aggressive personality with schizoid tendencies superimposed on a more or less marked mental deficiency (Zublin, 1953; de Pasqualini *et al.*, 1957; Raboch and Sipova, 1961). Pathological psycho-sexual behaviour is sometimes noted.

Various studies have added to this information.

In three hospitals for psychopaths ten males out of 1625 were detected by their positive chromatin (Forssman *et al.*, 1963); the striking thing about them was the wide variety of mental disturbances, confusional, episodic, schizoid, paranoiac and obsessional disorders, manic depressive psychoses and epilepsy.

The EEG results were for the majority abnormal confirming the fact already noted by Dumermuth (1961) in 14 chromatin-positive boys.

Association with phenylketonuria has also been observed (Benirschke *et al.*, 1962e).

It is therefore not surprising that detection of these patients in institutional populations has proven fruitful. It is also interesting to note that inquiries carried out in different regions with the chromatin criterion among mentally defective persons have given equivalent proportions of chromatin-positive subjects; 10 of 1252 (Mosier *et al.*, 1960a); 14 of 1506 (Barr *et al.*, 1960c); 9 of 916 (Ferguson-Smith, 1962); 28 of 2607 (MacLean *et al.*, 1962b), that is, in all 61 of 6281, or 9·71 per 1000.

These chromatin-positive subjects have usually one, two or three sex-chromatin masses and therefore probably represent XXY, XXYY, XXXY and XXXXY types with or without mosaicism. We find here the diversity observed in the newborn even though a complete karyotypic comparison is not yet possible. MacLean *et al.*, however, found a frequency of XX/XXY mosaics in one of the inquiries among newborn (1961) in 4 of 3000, quite close to that which they obtained for backward subjects at 2 out of 2607 (1962b).

If we consider the overall results of the chromatin criterion, 2·06 per 1000 chromatin-positive male newborn infants and 9·71 per 1000 in mentally defective subjects, the comparison brings out an association between gonosomal aneuploidies in subjects of male phenotype and mental deficiencies which it is difficult to attribute to chance alone. This comparison presupposes that the risk of pre-pubertal mortality is equal in both groups. If it is greater for the group of mental defectives institutionalized usually after 15 years, the ratio 9·71/2·06 underestimates the real position.

After Smithells (1962) had noted psychopathic or even criminal tendencies among some individuals with Klinefelter's syndrome, the proportion of chromatin-positive subjects was investigated (Wegmann *et al.*, 1963) in a population of 1318 deliquent or criminal adolescents or young adults. Only two subjects were detected or 1 in 658. They were suffering from Klinefelter's syndrome. This proportion is less than that indicated above of 2·1 per 1000 calculated for 6801 male neonates. It therefore does not appear that this chromosomal aberration increases juvenile deliquency at the same rate as mental backwardness.

To these findings we may add those gathered by Forssman *et al.* (1963) in three criminal asylums. Of 760 they found 15 chromatin-positive subjects or 1 in 50. This result is greater than that in the inquiries conducted among adult mental defectives at 9·5 per 1000. In schools for educable mental defectives the proportions of chromatin-positive boys are very variable (Prader *et al.*, 1958; Ferguson-Smith, 1959; de la Chapelle and Hortling, 1960; Cornwell, 1961; Israelsohn and Taylor, 1961). This disparity by country contrasts with the agreement in the inquiries among the newborn and is perhaps due to different educational systems.

(d) Clinical evolution

(i) The XXY newborn or even the child under 1 year may be indiscernible, even with testicular biopsy, without resort to chromatin and karyotypic criteria by which it is now possible to detect XXY infants.

A hypotrophic form has been recognized with reduced weight and stature and retardation of osseous maturation (Alexander *et al.*, 1963).

A sublethal form with cardiopathy was revealed by systematic inquiry. Among 104 male children with congenital cardiopathies were two 7-month XXY infants with tetralogy of Fallot (Gautier and Nouaille, 1963).

(ii) The apubertal peno-testicular peculiarities have particularly drawn attention to this condition.

Cryptorchidism and hypospadias are not noted in general among adult subjects with Klinefelter's syndrome (Bishop and Polani, 1960; Decourt *et al.*, 1962). On the other hand, in children vulviform hypospadias have sometimes been reported (Reifenstein, Jr., 1947; Klotz and Sors, 1958; Lelong *et al.*, 1959; Lamy *et al.*, 1963b), and also bilateral cryptorchidism (Mozziconacci *et al.*, 1962) or peno-testicular hypoplasia.

An observation of Gray (1961) reports the coincidence of these two malformations in a 5-year-old boy with positive jugal chromatin and of XXY karyotype (blood): peno-scrotal hypospadias, left scrotal testicle, but right inguinal testicle. The author of this communication quotes a further example of hypospadias (Halbrecht, 1960) in a chromatin-positive newborn infant. He mentions disturbance in urethro-peno-scrotal differentiation with anomalous androgens of the primary gonad (Jost, 1950).

It is possible that desire for therapy brings children with these anomalies to the paediatrician since certain inquiries do not indicate that their frequency in XXY newborn infants (MacLean *et al.*, 1961) is higher than in XXY adults.

However, since hypospadias and cryptorchidism may rightly be considered as signs of intersexuality rather than of Klinefelter's syndrome, such a coincidence in any event justifies search for a mosaicism.

Although the characters of the testicle of Klinefelter's syndrome in the adult are well defined, the description of those in a child runs up against the imprecisions inherent in testicular histology at this age (Lawrence and Yuceoglu, 1961). The testicles of the prepubertal subject with Klinefelter's syndrome are normal, apart from poverty of the germ cells. The onset of the degenerative lesions is uncertain but peritubular fibrosis may appear from the age of 7 years. Tubular hyalinosclerosis is not apparent before the age of 12 years.

(iii) Puberty is retarded, incomplete and pathological. Osseous maturation contributes to this retardation. Development of the secondary sex characters, particularly testicles, and of sex hair does not occur. The signs of XXY proper come to the fore giving a pathological aspect to this type of puberty with gynaecomastia, tubular hyalinosclerosis, increased gonadotrophins in the urine and frequent retardation of mental awakening.

(iv) With time, the XXY adults are predisposed to certain morbid processes reported in a still small number of cases (Lubs, 1962; Rohde, 1963b). Impair-

ment of the osteo-muscular apparatus is, in the view of certain workers, a sign of early deficiency of Leydig cells. This deficit is thought to produce osteoporosis, sometimes complicated by kyphoscoliosis and muscular weakness sometimes with inguinal hernias.

Renal lithiasis, cancer and, especially, chronic pulmonary emphysema are often noted. Finally, certain diseases sometimes noted in the family of XXY subjects also affect them, in particular, cerebellar degeneracy in varied forms (Hecht and Ruskin, 1960; Indemini and Amman, 1961). More cases are necessary to complete this interesting pathology of older XXY subjects.

(e) Clinical forms

The karyotypic criterion leads one to relate the XXY type to varieties which are distinguished from it by some physical or functional peculiarities. This is a matter less of the eunuchoid form than of the form suggestive of "germinal aplasia" and alleged fertile XXY subjects. Cytogenetics by detecting many cases of coincidence of XXY with other chromosomal aberrations has opened up the important chapter of associated forms.

(i) The eunuchoid form of the adult figured in the initial description of Klinefelter's syndrome. It is distinguished by insufficiency of the secondary sex characters, of the Leydig tissue and the 17-ketosteroids level. Then a case was reported with positive chromatin and large accumulations of Leydig cells (Greenblatt, 1958).

A paper by Decourt (1960a) shows that the karyotypic criterion does not justify a distinction between the classical variety of Klinefelter's syndrome and the eunuchoid variety. In a 51-year-old chromatin-positive subject the physical features of his small testicles with epididymal tissue and urinary gonadotrophin level above 100 mouse units suggested a Klinefelter's syndrome. But in this macroskele subject measuring 1·80 m hypertrophy of the penis with hypotrichosis was barely corrected by hormone therapy. In addition, gynoid adiposity with adipomastia had developed without genuine gynaecomastia. The testicular histology was such that under a thickened albuginea and among the sclerous and hyalin tubes without a seminal or Sertolian content it was possible to discover only rare eosinophilic interstitial cells. Absence of Leydig hyperplasia in the clinical context clearly distinguished this patient from the initial classical variety. Chromosome examination and an XXY constitution brought him nearer to this variety.

(ii) A subject with positive chromatin and male phenotype may be mentioned here in view of his probable XXY karyotype and the peculiarities of his testicular dysgenesis (Laguens *et al.*, 1961). This was expressed in low gonadotrophin level, absence of spermatogenesis, the thickening of the basement membrane without hyalinosis, integrity of the Sertoli cells and reduction in the Leydig cells. These features are quite suggestive of those of "germinal aplasia" described by del Castillo *et al.* (1947a) particularly since the form with positive chromatin was reported. However, the deviant features claimed the attention of the authors, not so much the small stature, webbed neck, broad thorax and cubitus valgus

which gave to this subject a Turner's aspect, was the discovery of a chromosome 1 with discrepancy of its arms: 1·5–1·75 instead of 1·1–1·2.

Finally, examples of XY "germinal aplasia" have been published (p. 196).

(iii) The problem of the fertility of XXY subjects has still not been solved but that it is possible is suggested by certain observations (Frøland and Ulrich, 1963; Warburg, 1963; Kaplan *et al.*, 1963). But the two necessary proofs, probability of paternity and absence of mosaicism are difficult to supply. The subject described by Warburg although the estimation of his spermogram was only about 1/1000 of normal was said to have had two boys to a female of normal karyotype, one who died in 1952 from poliomyelitis, the other a 15-year-old normal XY. Probability of paternity from anthropological criteria (erythrocyte and serum phenotypes, pigmentation and dermatoglyphs) appears ten times greater than non-probability. The karyotype was made of the father with possible chromatin-positive Klinefelter's syndrome. It revealed a majority of XXY cells (blood and skin) without eliminating the possibility of an XY/XXY mosaicism (p. 213). Therefore, doubt remains on the part which the XY cells could have played in the development of the primary gonads. The same remark applies to the varied descriptions of testicles of Klinefelter's syndrome involving some tubes with mature spermatozoids.

The XXY mouse, the replica of this human type, retains the male phenotype. It is exceptional, sexually active but sterile (Russell and Chu, 1961; Cattanach).

(iv) The XXY subjects perhaps provide the largest number of observations of coincidence in the same subject of chromosome aberrations: XXY and trisomy 21 (Ford *et al.*, 1959c; Harnden *et al.*, 1960c; Lanman *et al.*, 1960; Lehmann and Forssman, 1960a; Van Gelderen and Hustinx, 1961; Hamerton *et al.*, 1961b; Milcu and Maicanesco, 1963a) and XXY and translocations (see below).

The most typical expression of these coincidences appears in two cases with presence both of trisomy 21 and Klinefelter's syndrome in monozygotic twins (Hustinx *et al.*, 1961; Turpin *et al.*, 1964c) (cf. Figs. 10.4 and 10.5).

These facts may again be compared with the concordance of certain traits peculiar both to trisomy 21 and Klinefelter's syndrome.

1. Their similar frequency according to the results of detection of XXY in newborn infants by the chromatin criterion.

2. The fall in the nuclear segmentation index (p. 61) may also be quoted (Turpin and Bernyer, 1947a; Mittwoch, 1961b). It is accompanied by a low figure for drumsticks. Although the sensitivity of these patients to acute leukaemias is not comparable to that in trisomy 21 the appearance of acute myeloid leukaemia has been reported in an XXY subject (Mamunes *et al.*, 1961). Another observation reports the existence of a formula interpreted as XXY/XY in the blood cells of a subject with presumed chronic erythraemic myelosis (Kemp *et al.*, 1961).

3. Translocations especially between acrocentrics have often been reported both in trisomic 21 and XXY subjects or in their parents or relatives and not merely translocations involving chromosome 21. In one case (Moorhead *et al.*, 1961) a translocation 22 ~ D existed in the mother and some sibs of a classical

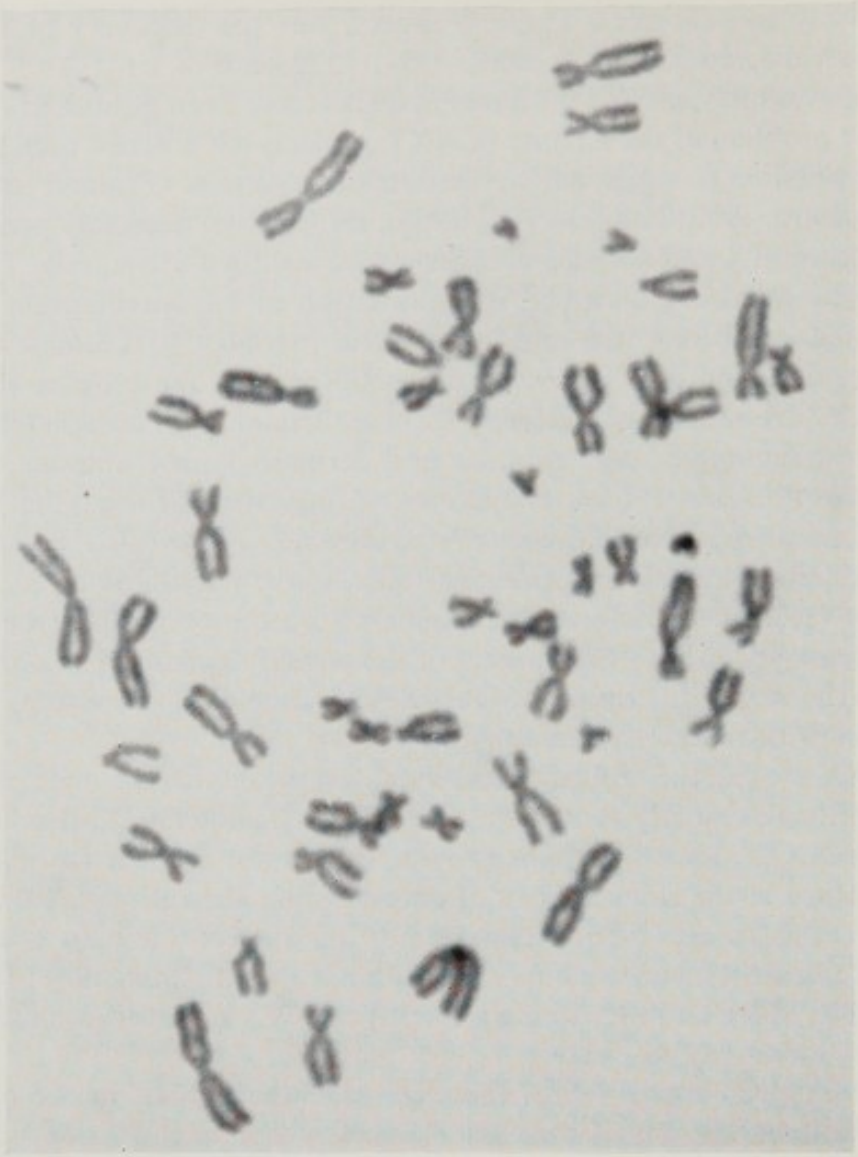


FIG. 10.4. Cell with 48 chromosomes. Klinefelter's syndrome combined with trisomy 21.

trisomy 21 with 47 chromosomes. In one of our cases mentioned above the paternal translocation 22 ~ D had been transmitted to the son who also had an XXY formula (Institut de Progénèse, No. 293).

A general phenomenon of chromosomal interaction, a first anomaly of a cell line increasing the probability of a second, finds its best examples in trisomy 21 and Klinefelter's syndrome.

4. Since the older the mother the higher the frequency of dizygotic and even monozygotic twinning, and the frequency of trisomy 21 and even of Klinefelter's syndrome (see above), it is possible that it is in part a common determining factor, but the problem is more complex. Twins, for example, are abnormally frequent in X0 and X0/XX families (Lindsten, 1963a) and yet the influence on the appearance of a Turner's syndrome of maternal age, though it has been suggested, has not been confirmed.

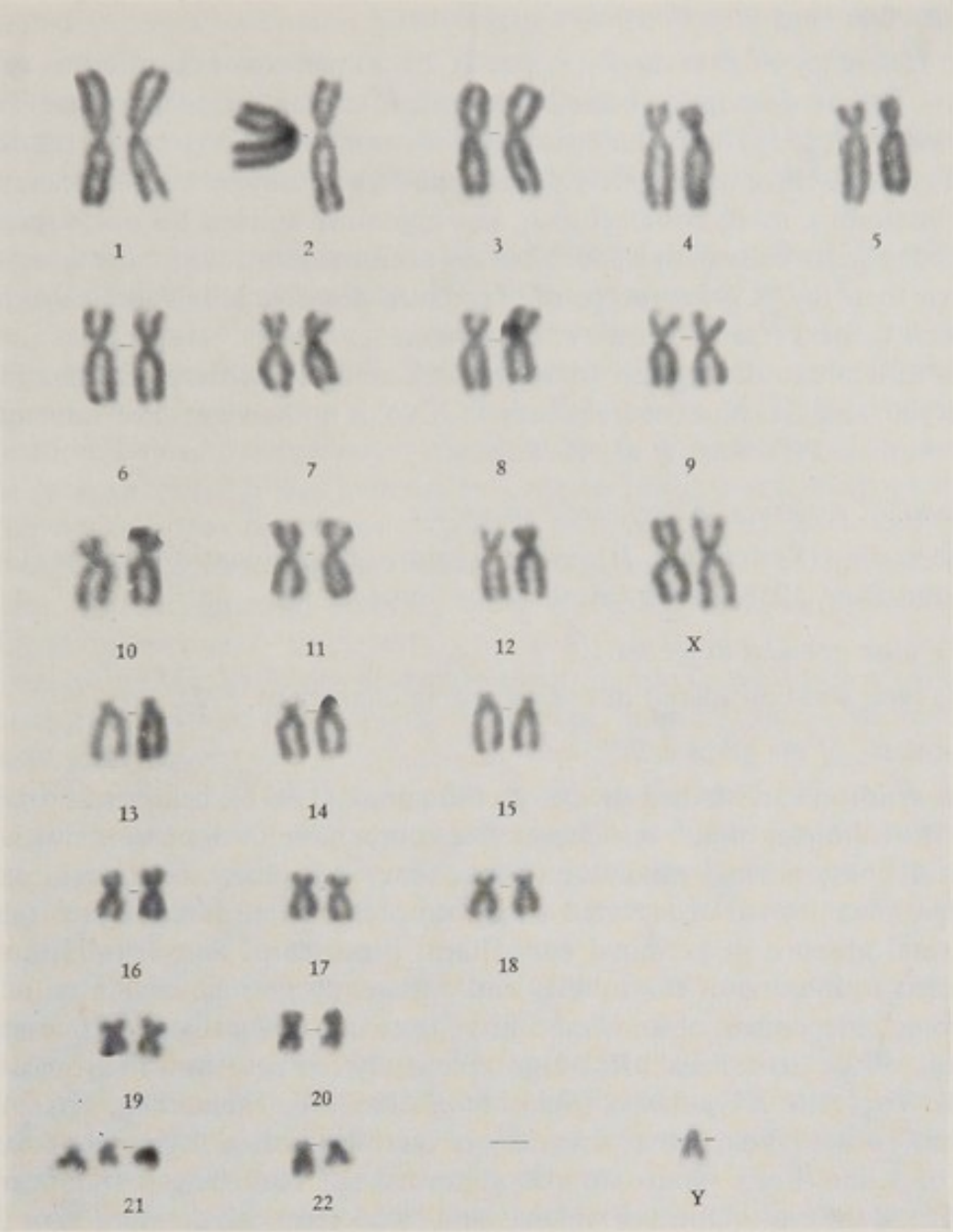


FIG. 10.5. Karyotype with 48 chromosomes, XXY and trisomy 21.

II. XY testicular dysgenesis

Various forms of testicular dysgenesis more or less close to Klinefelter's syndrome are gradually emerging from the confused picture. Karyotypic analysis permits two groups to be distinguished.

On the one hand, XY subjects, including chromatin-negative subjects with Klinefelter's syndrome.

On the other, karyotypic varieties without mosaicism for example, XXXY and XXYY, or with mosaicism, varieties considered later in this book. The necessary work is gradually being done to achieve anatomo-clinical individualization of each of these varieties.

(a) Chromatin-negative Klinefelter's syndrome

The frequency of this variety is put at 20–50 per cent according to various authors. From a description preceding the use of the karyotypic criterion (Grumbach and Barr, 1958) testicular lesions of this variety appear to be less than those of the positive-chromatin variety. The seminiferous tubules would appear to be more numerous, more homogeneous, less immature and less fibrous, sometimes containing germ cells with smaller Leydig accumulations with normal cells.

Since then the XY karyotype of chromatin-negative Klinefelter's syndrome has been verified (Court Brown *et al.*, 1960).

It is difficult to distinguish between XXY and XY without chromatin and karyotypic analyses and the frequency of XXY is in the view of certain authors underestimated (Stewart *et al.*, 1959e).

(b) Familial variety of Klinefelter's syndrome

This variety (Reifenstein, Jr., 1947) is said to be chromatin-negative (Grumbach and Barr, 1958) and transmitted by females.

(c) XY pure gonadal dysgenesis

This type was considered in the preceding chapter (p. 172).

(d) "Aplasia of the germ cells"

This syndrome, described by Del Castillo *et al.* (1947a), brings with it in the adult the following signs: testicles of size comparable in dimensions with pre-pubertal ones; normal phallus and secondary sex characters; sterility with azoospermia; normal or elevated gonadotrophins; small seminiferous tubules with total absence of germinal epithelium; presence of Sertoli cells; normal basement membrane of the tubules and apparently normal Leydig cells. This syndrome independent of known acquired testicular lesions is sometimes called "aplasia of the germ cells". Its karyotypic study has not shown chromosomal lesions. These are XY subjects (Solari *et al.*, 1963; Valencia *et al.*, 1963b).

It has been suggested that a variety of sterility with XY formula covering cases of Klinefelter's syndrome and abnormal spermatogenesis (Klotz *et al.*, 1963b) and the typical form of which is said to be germ cell aplasia (de la Balze *et al.* 1963a) is thought to have a genic origin. Its animal equivalent is said to be hereditary sterility of the male mouse (Mintz, 1960).

(e) "Male XY Turner's syndrome"

Since 1943 (Flavell, 1943) observations have been published on male subjects with negative chromatin whose appearance and malformations were very evocative of Turner's syndrome (Steiker *et al.*, 1961; Fraccaro *et al.*, 1961c; Court Brown *et al.*, 1960a). More than 30 examples of these male variants of this syndrome have been published. The karyotypes of about one in three have been established; they are all XY. An observation at first considered as XX (Oikawa and Blizzard, 1961) was identified as a mosaic including a cell population with an abnormal Y (p. 291) when a further study was made (Solomon *et al.*, 1963).

Most of these forms of dysgenesis have been identified in infants or older but pre-pubertal subjects. Three examples (Chu *et al.*, 1961b) illustrate the characters

of the first variety shortly after birth; oedema of the back, hands and feet, hair low set and cutis laxa in the nape of the neck, short neck, low setting of ears, bifid thumb, cardiopathy and masculine genital organs and scrotal testicles. The blood cultures of one of these infants showed of 50 cells 39 with 46 chromosomes. After a discussion the authors rejected a mosaicism and favour an XY formula.

The older but still pre-pubertal child lends himself to more comprehensive study (Morishima and Grumbach, 1961; De Gennes *et al.*, 1963a).

The basic physical signs are dwarfism with retarded osseous maturation and very frequently webbed neck. Differing heart conditions are reported in about one in five, with pulmonary stenosis, aortal shrinkage or interauricular communication. Gonadal dysgenesis is characterized by the absence of any element of the spermatogonal line. There are few or small calibre seminiferous tubules and Leydig cells may be completely absent, even in subjects previously treated with chorionic gonadotrophin, features found in scrotal or intraperitoneal testicles. The cryptorchid testicles were studied before the appearance of modifications secondary to puberty.

Apart from these basic physical signs the unsightly face (ptosis, nasal notch, hypertelorism, low-set ears), shield-like thorax, cubitus valgus and naevi are more or less frequent.

The hormonal signs of this gonadal dysgenesis go hand in hand in certain cases with retardation of puberty: gonadotrophins less than 3 mouse units show despite gonadal dysgenesis, very weak gonadotrophic stimulation. The suprarenal glucocorticoid function has been found normal contrasting with insufficiency of androgenic function.

The psychic signs include retardation of psychomotor development noted in about one in three (Futterweit *et al.*, 1961).

The origin of masculine XY gonado-somatic dysgenesis is unknown. The hypothesis of a gene mutation has been advanced, tentatively postulating a chromosomal aberration which may escape present cytogenetics. But this hypothesis is not supported by any precise argument.

(f) Certain varieties of hypogonadism acquired during infancy may produce a phenotype very similar to that of XXY. These acquired XY varieties originate from lesions through inguinal surgical interventions, ionizing radiations and bilateral orchitis (mumps). Others have been observed following cortical or hypothalamic encephalitis and skull injuries (Sendrail, 1962a). Other hypogonadisms are distinguished by the characteristic signs of the causal disorder: hypopituitarism, anhydrotic ectodermal dysplasia, progressive muscular dystrophy (Erb) and Laurence–Moon–Biedl syndrome.

As for the testicular complications of myotonic myopathy (Steinert's disease) they are late and preceded by a period of fertility. The sclerous and hyalin involvement of the tubules is accompanied only very irregularly by Leydig hyperplasia and increase in gonadotrophins. Chromatin is negative (Decourt, 1960a) apart from a case suggesting the possibility of an association.

(g) The karyotypic varieties described in the following pages give phenotypes more or less suggestive of Klinefelter's syndrome.

XXXY type

The detection in 1959 (Barr *et al.*, 1959 b, c) among male mentally handicapped persons, of two subjects with two sex chromatin masses suggested that they must be equipped with several X chromosomes, even four, since one mass was then considered to be the result of association of the heterochromatic segments of the two X chromosomes.

The following year, Ferguson-Smith, Johnston and Handmaker (1960e) reported two male subjects with two sex-chromatin masses in a large proportion of nuclei and with an XXXY formula. This publication not only isolated a new chromosomal type but confirmed the opinion of Ohno, Kaplan and Kinosita (1959) and Ohno and Makino (1961c) on the nature of the chromatin body (pp. 278 and 280). It indicated that the number of nuclear chromatin bodies is equal to the number of X chromosomes minus one.

The two subjects who made possible isolation of this XXXY variety were detected in an institution for the mentally backward. Signs of Klinefelter's syndrome constituted their most conspicuous feature.

(a) Their morphology was very comparable, tall subjects with small head and proportionately too long legs.

The first whose skeletal age was 17 years for a chronological age of 22 years had a narrow chest, lumbar lordosis and sparse hair other than pubic. His secondary sex characters were those of an adolescent but his testicles palpable on each side in the scrotum were very small and firm, and his prostate was also small.

The second, though he appeared younger than his age, did not suffer from retardation of skeletal maturation. His pelvis was android. His hair cover was sparse apart from the pubis and axillas. His secondary sex characters were those of an adolescent with well-developed larynx. His very small testicles were perceived with difficulty at the root of the sacs; the prostate was small.

They showed certain other striking features with pterygium coli, radio-ulnar synostosis and absence of gynaecomastia in the first case and absence of webbed neck and synostosis but presence of gynaecomastia in the second.

(b) The mental state of these two subjects was very poor. The mental retardation of the first appeared during growth; at 22 years his IQ (Wechsler) was 49. The second at 9 years had already shown considerable backwardness, at 22 years his IQ (Wechsler) was less than 20.

(c) The testicular histology of these two subjects was concordant. Whereas the epididymus and vas deferens were well developed, the length of the testicles did not reach 2 cm. The seminiferous tubules for the most part were completely atrophied and hyalinized and lost in an amorphous connective stroma interspersed with wide islets of abnormal and pigmented Leydig cells. Very rare tubes were fringed by Sertoli cells but without the slightest trace of germ cells. Some of these tubes were partially hyalinized. Some of the smallest arterioles of the second subject were also affected by hyalinization.

(d) Jugal chromatin in both was similar (about 36 per cent cells with one and 40 per cent with two masses), not accompanied by parallel changes in the drum-

sticks. The features of the latter in XXXY subjects therefore appear to be comparable with those in XXX subjects (p. 281).

(e) The karyotypes established from bone marrow samples were similar. Of 153 cells of the first, 116 had 48, 13 had 47, 11 had 46 and 10 had 45 chromosomes. Of 118 cells of the second, 87 had 48, 9 had 47, 13 had 46 and 8 had 45 chromosomes. Karyotypic analysis of the cells with 48 chromosomes, 15 for the first, 5 for the second, gave an XXXY formula.

(f) Various meiotic or mitotic non-disjunctional hypotheses may explain the production of an XXXY constitution (p. 271). Familial inquires have provided few indications so far on these mechanisms.

The first subject had nine normal brothers and sisters. His chromatin-positive mother with one chromatin body therefore was not XXX. The father was dead. One of the sisters of the patient had a hydrocephalic son with multiple deformations.

The second subject came from a family whose paternal branch was heavily tainted: his father, uncle, grandmother and a cousin were schizophrenics. His mother was chromatin-positive with one mass, the father chromatin-negative. The chromatin criteria in both cases justified the conclusion that these XXXY subjects were not the sons of an XXX mother; and for the second that he was not the son of an XXY father, a highly improbable hypothesis in view of the practically unchallengeable sterility of XXY subjects.

A subsequent independent study (Carr *et al.*, 1961 b) was to enrich this description with two new cases.

The first concerned a 15-year-old boy of normal appearance, apart from small testicles, with IQ of about 60. Jugal and skin chromatin was positive and 30 per cent of the positive nuclei had two masses. The urinary signs and testicular histology were characteristic of chromatin-positive Klinefelter's syndrome.

The second was a 14-year-old boy of normal appearance apart from small testicles and slight bilateral gynaecomastia, his IQ (Wechsler) did not exceed 60. Jugal chromatin was positive with one body in 33 per cent of the nuclei and with two masses in 42 per cent. Skin and Leydig chromatins gave a comparable but a little higher proportion. The results of the urinary and testicular examinations were characteristic of Klinefelter's syndrome.

For both subjects qualifications must be made on the thyroid functions (low values of basal metabolism, fixation of ^{131}I, response to thyreotrophin).

Chromosome analysis of the first subject was made for the blood and with two skin biopsies. In all, of 90 cells, 80 had 48, 9 had 47 and 1 had 49 chromosomes. The XXXY formula was verified in many karyotypes of the cells with 48, on the other hand, cells with 47 resulted from the absence of a different chromosome for each, the cell with 49 was the result of an artefact.

The second subject was studied by sampling of blood, bone marrow and four skin biopsies. In one of the skin cultures an isolated anomaly was identified, resulting from the rapid proliferation of an atypical clone. The XXXY formula was verified in five other cultures.

Another observation (Breakey, 1961), found by sex-chromatin analysis of a group of 297 backward subjects including 157 males, is also typical of a Kline-

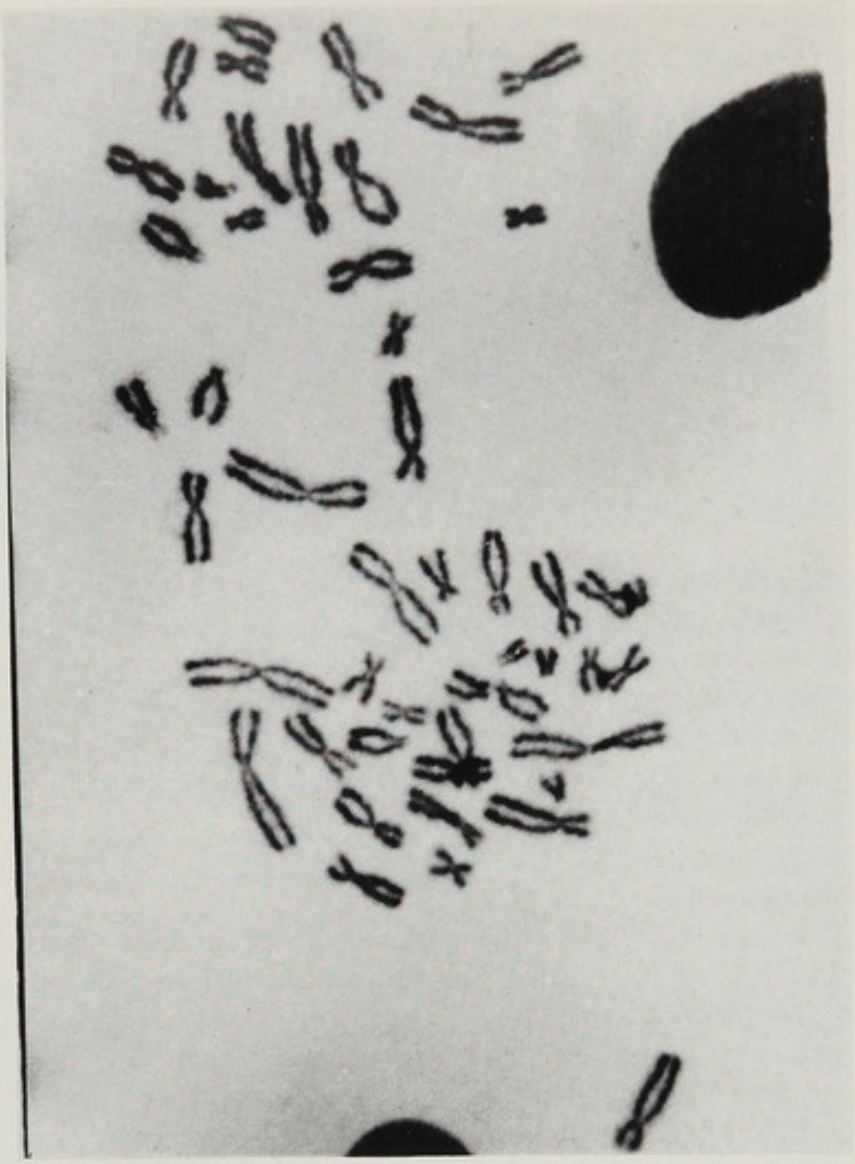


FIG. 10.6. Cell with 48 chromosomes. XXXY gonadal dysgenesis.

felter's syndrome. However, few degenerated seminiferous tubules and many interstitial cells were noted during examination of the two testicles. Of 700 jugal cells 37 per cent had one mass and 7 per cent two of XXXY karyotype. Chromatin study of the father, mother and six sibs showed no anomaly. The patient suffered from partial colour blindness and the Ishihara test revealed in the mother alone a lesser but real defect of perception.

The author of this observation assumes that the XX of the mother carried one or two mutants responsible for partial colour blindness, that an XXX ovum resulting from a meiotic non-disjunction carried two or three mutants and that this supplementary dose produced in the XXXY child a more accentuated disturbance in perception than the maternal one.

All these observations concern subjects with considerable or even serious mental backwardness. It would be premature to deduce from them that accentuation of backwardness is, in presence of an extra X, the feature which

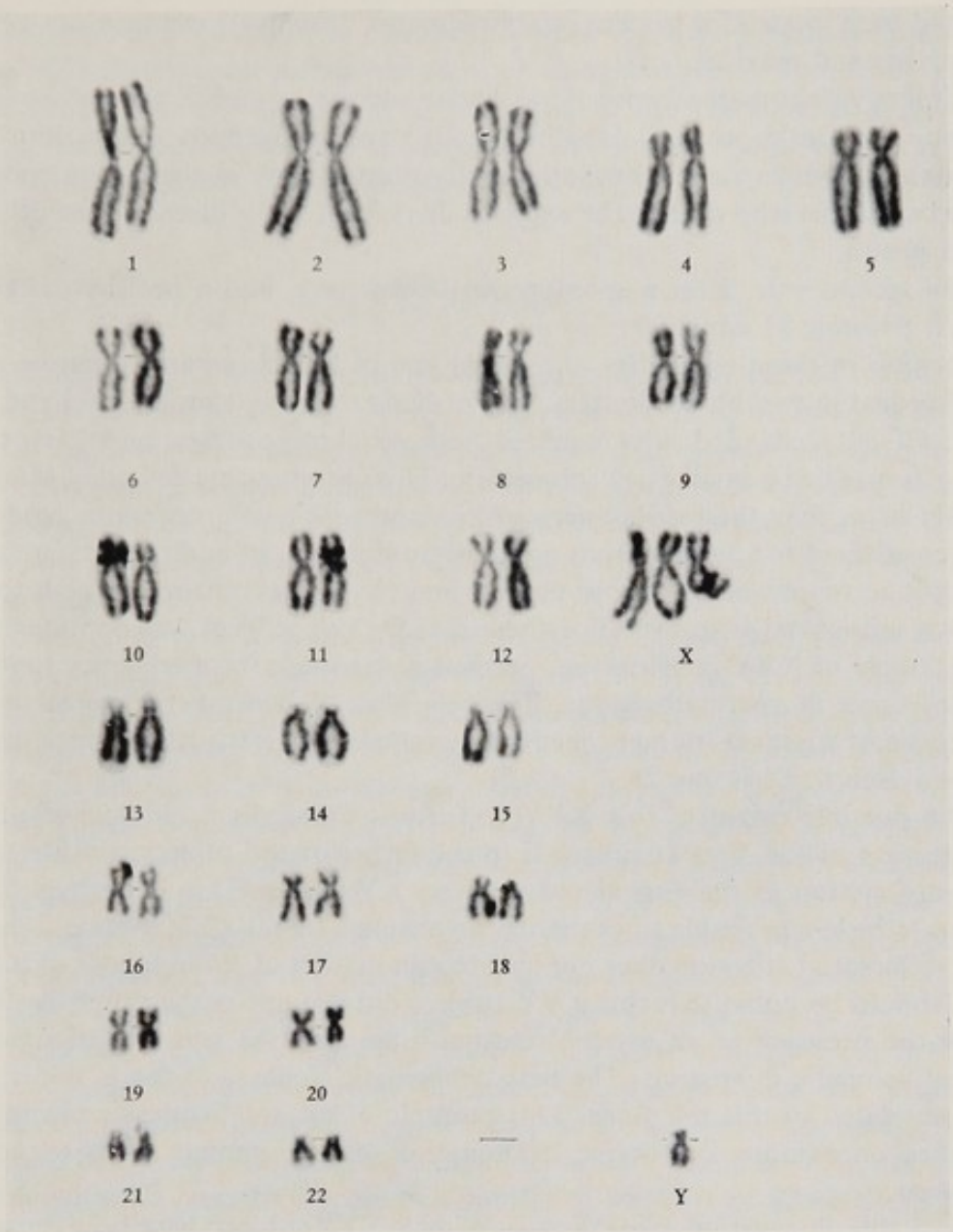


FIG. 10.7. Karyotype with 48 chromosomes. XXXY gonadal dysgenesis.

distinguishes these subjects from XXY subjects with Klinefelter's syndrome. In fact, the above observations were all detected among subjects admitted for mental disturbances.

XYY type

The subject who provided the occasion for the description of the XYY type (Sandberg *et al.*, 1961b; Hauschka, 1962b) attracted attention less by his physical and psychic features proper than by the anomalies of his progeny.

He was a corpulent male aged 44 years of average intelligence and neither his appearance nor his past indicated an abnormal chromosomal constitution.

He had been treated for hernias (diaphragmatic, umbilical), peptic ulcer, neurodermatitis and maxillary cyst.

His first wife, apparently psychotic, had in succession an XX primary amenorrhoeic, chromatin-positive daughter, with ovarian agenesis (laparotomy); a spontaneous miscarriage; three apparently normal boys in succession and two male twins, one who died at the age of 3 days from "blue disease", the other in good health.

The second wife, after a spontaneous miscarriage, had a healthy daughter, then a trisomic 21 daughter.

Because of these anomalies, the karyotype of this chromatin-negative male was studied in two blood samples, two of bone marrow (sternal, iliac) and one skin. Of 301 cells, 263 were counted with 47 chromosomes, and karyotypic analysis revealed a small extra acrocentric. This chromosome devoid of satellite, a little larger than the smallest acrocentrics and with a heteropycnotic tendency was considered for these reasons as a Y chromosome. In addition, its presence was not accompanied by serious psychic and physical anomalies peculiar to the known autosomal trisomies. For these reasons this subject was considered as an example of XYY constitution, perhaps responsible for a tendency towards disturbances in spermatogenesis. The relations of these disturbances to the ensemble of stigmata in the progeny deserve mention, particularly since one of the children had trisomy 21.

The possible origin of this XYY constitution was discussed: meiotic non-disjunction of the Y at anaphase II in the father of the subject considered or non-disjunction in the first cleavage of an XY zygote. This latter hypothesis seems to be less probable since there is an example of X0/XYY mosaic showing that differential selection does not lead to elimination of X0 in favour of XYY.

It should be noted that this XYY subject did not appear to suffer ill-effects from the presence of an extra Y chromosome since he was free of signs of gonado-somatic dysgenesis. The heterochromatic richness of the Y is perhaps not unrelated to this tolerance. This example of an uninformative phenotype justifies once more karyotypic examination of the parents of trisomic 21 children.

Since this observation, two examples of male XYY subjects have been reported by Fraccaro *et al.* (1962b), distinguished from the preceding case by their mental deficiency and absence of descent of the testicles.

A case at first described as trisomy 22 responsible for the Sturge-Weber syndrome (Hayward and Bower, 1960b) was recently recognized as a further example of XYY (Dent *et al.*, 1963a) by blood and fibroblast cultures. This observation concerned a mentally defective boy aged 3 years 10 months with a cranial perimeter of 0·48 m and frank neurological signs: convulsions, hyperkinesis, retardation of psycho-motor development and of speech, with slight left hemiparesis. On the right side there was buphthalmia, a skin naevus in the region of the ophthalmic and maxillary branches of the trigeminal and extensive right parieto-occipital calcification with typical double contoured "gyral" arrangement.

Other examples are accompanied by genital anomalies. One was complicated

by a hypospadias (Milcu *et al.*, 1963b); another by hypogonadism (Buckton *et al.*, 1963); hypogonadism was the most prominent aspect of another detected in a 25-year-old male with penis and scrotum of the pre-pubertal type, and with no normal development of the testicles. This subject had as well an equine varus type of club foot (Court Brown *et al.*, 1964).

XXYY type

Although his gonosomal anomaly was apparently balanced, the 15-year-old boy observed by Muldal and Ockey (1960) was a typical example of Klinefelter's syndrome with mental retardation. The discrepancy between the exceptionally high gonadotrophins and the low 17-ketosteroids was very considerable and jugal chromatin was positive with drumsticks. The bone marrow which alone was examined revealed an XXYY karyotype (87 per cent cells with 48 chromosomes).

A few months later a 52-year-old male, a new example, was detected by his positive chromatin in a centre for oligophrenics (Carr *et al.*, 1961e). At 31 years his IQ (Stanford–Binet) was 38. The gonado-somatic signs and urinary hormonal excretions were typical of Klinefelter's syndrome but with the XXYY karyotype. The testicular histology on each side was distinguished by the appearance of poorly organized connective tissue, the degeneration of Leydig cells grouped in a few islets and the rarity of seminiferous tubules with a thickened basement and Sertolian epithelium. A fragment of epididymus was normal. To explain this histological type the authors refer to the possible secondary role of a regressive process in relation to the age of the subject. This patient was the product of a dizygotic twin pregnancy, the other twin was normal.

A third case (Ellis *et al.*, 1961b) ties up with these by karyotype. It again concerns a tall mental defective with acromegaloid appearance and testicular atrophy complicated on the left by absence of the greater part of the epididymus.

Mental deficiency is also noted in the observation (Vague *et al.*, 1961), comparable with the preceding ones, of a subject of 35 years of Klinefelter's syndrome clinical type, with extensively or totally hyalinized seminiferous tubules and Leydig hyperplasia. The XXYY formula was found from study of the skin.

The clinical type of two subjects, one 28, the other 18 years, detected in an institution for mental defectives (MacLean *et al.*, 1962b) was also suggestive of Klinefelter's syndrome.

A confirmation of these features, mental deficiency, IQ 35 and habitus of Klinefelter's syndrome is again given by the observations on a 48-year-old eunuchoid chromatin-positive subject with presence of drumsticks and XXYY in blood (Laurence *et al.*, 1963b). This subject was distinguished by ocular disturbances: strabismus, bilateral cataract, choroiditis and hypertrophy of the frontal sinuses; marked increase in the "serum mucoproteins" and a value of urinary chondroitin sulphate at the upper normal limit.

Three other cases have since been reported by Barr *et al.* (1964) and another by Stimson (1964).

These observations show that an extra Y cannot bring back into balance the 2AXXY genotype responsible for the chromatin-positive Klinefelter's syndrome.

These subjects are often eunuchoid with gynaecomastia, they sometimes suffer, when they are old, from varicose veins of the lower limbs with skin ulcerations.

A newborn child detected from the chromatin criteria had a normal appearance (Court Brown *et al.*, 1964).

XXXXY type

In October 1960, Fraccaro, Kaijser and Lindsten reported on a boy with gonado-somatic dysgenesis. They interpreted his karyotype with 49 chromosomes as an association of an XXY formula and a double somatic trisomy (8 and 11). On the following 10 December after finding chromatin-positive jugal cells with three masses they abandoned the previous interpretation for that of an XXXXY (Fraccaro and Lindsten). This discussion suggests the possibility of significant variations in the dimensions of the X chromosomes (Böök, 1961a).

Three days earlier on 7 December, Anders, Prader, Hauschteck, Schärer, Siebenmann and Heller had submitted for publication the case of a child aged $8\frac{1}{2}$ years with considerable psycho-motor retardation and gonado-somatic dysgenesis, a probable example of mosaic but with a majority of XXXXY cells in 36 cells (bone marrow), 22 XXXXY cells, two XXXXXY, one XXX and the others with 48–50 chromosomes but with one or two non-classifiable autosomes. At present, 15 observations of subjects with a single or predominant XXXXY clone (blood, skin, fascia lata) have been reported (Fraccaro *et al.*, 1960g,b; Anders *et al.*, 1960b; Fraser *et al.*, 1961; Miller *et al.*, 1961b; Barr *et al.*, 1962c; Fraccaro *et al.*, 1962e; Pfeiffer, 1962b; Thuline *et al.*, 1962; Turpin *et al.*, 1962b; Atkins and Connelly, 1963a,b; Day *et al.*, 1963c; Lamy *et al.*, 1963a; Schade *et al.*, 1963a,b; Prader *et al.*, 1964). Some of these observations similar in anatomo-clinical features are mosaicisms, particularly XXXY/XXXXY but with predominance of the XXXXY clone (Anders; Lamy; Prader). Mosaicisms with minority XXXXY clones are mentioned elsewhere (p. 214).

A personal observation (Turpin *et al.*, 1962b) enabled us to analyse an XXXXY type at the age of $3\frac{1}{2}$ months.

From the outset the clinical features of the child with this gonado-somatic dysgenesis were conspicuous. The primigravid pregnancy disturbed during the first 4 months by vomiting, had been accompanied in the last 5 by a gain in weight of 17 kg. The child was born at term, weak, weighing 2460 g and was declared to be of male sex. The father and mother were then both 25 years old.

At $3\frac{1}{2}$ months notable microcephalia, low and narrow forehead, rounded and protruding face, nasal notch, moderate hypertelorism, low-set ears and retrognathism suggested a developmental anomaly which the features of the external genitals confirmed. The penis, less than 1 cm long but with normal urethral orifice, was lodged in the interscrotal fissure and the sacs on both sides of a very wide raphe had a pseudo-labial appearance. However, they retained their transverse folds. In the scrotal position, near the impenetrable inguinal orifice,

a mass the size of a lentil and of testicular consistency was palpated on each side. At the age of 4 months radiological and surgical exploration was made. Retrograde urethrography established a masculine urethra with very short anterior urethra and in the posterior urethra the picture of veru montanum.

The radiograph already showed clinodactyly of the fifth finger, shortness of the first metacarpal and a certain widening of the proximal extremity of the ulna. Laparotomy verified the absence of any feminine even rudimentary formations. Right scrotal exploration confirmed a minute testicle with its normal appendages.

The testicular preparations revealed in a loose stroma many seminiferous tubules of normal size and without cell differentiation. There were no Leydig cells in the interstitial tissue. The appearance was that of a non-puberty testicle. Urinary excretion at 4 months and at 7 months of the 17-ketosteroids, 17-hydroxysteroids, pregnanediol, pregnanetriol and gonadotrophins did not diverge from the normal values for this age. That of the oestrogens, particularly at 4 months, was higher than anticipated. The jugal chromatin studied in 1000 cells was found positive with one mass in 60 cells, two in over 200 and three in a few sometimes with bipartite elements.

Chromosome study in 200 (peritoneum) and 11 (testicles) cells gave 49 chromosomes in all of them; karyotypic analysis revealed a 44AXXXXY formula without evidence of mosaic.

As this child developed during the first year of life, his hypotrophy with microcephaly, incipient webbed neck and his psycho-motor retardation became more accentuated. At the age of 7 months, his developmental age was 5 months for postural control and oculo-motor co-ordination, 6 months for sounds and sociability, 3½ months for EEG; at 5 months on the occasion of an infectious state several convulsive seizures appeared.

The dermatoglyphic study could be made more precise at the age of 19 months. It gave the following results: practically normal palmar prints, index of transverse ridges on left 26, on right 31; axial triradius in the intermediate position (t') on both sides.

Fingerprints; from the 1st to the 5th finger in order—on the right: UL, A, UL, UL, UL; on the left: UL, A, A, UL, UL (A, arch, UL, ulnar loop). The "arch formation" was therefore found only three times.

The non-consanguineous parents of this child were normal. Their karyotypes were free of numerical or structural anomalies. Cousins of the child were normal apart from the son of one of his paternal uncles who had been operated on at birth for anal imperforation (?) but who was free of any other anomaly.

At 2 years 6 months this small patient struck one by his indifference, his motor retardation with hypotonia (did not stand up), cranial perimeter of about 46·25 cm, height 0·81 m and weight 9·600 kg. Urinary biological oestrogens were normal.

If we now consider the features of the 15 observations mentioned above it is possible to isolate the main traits of this XXXXY type. Its karyotypic study is in general justified by the presence of more or less numerous cells with three chromatin masses mixed with those with one and two masses. The use of ^{3}H-

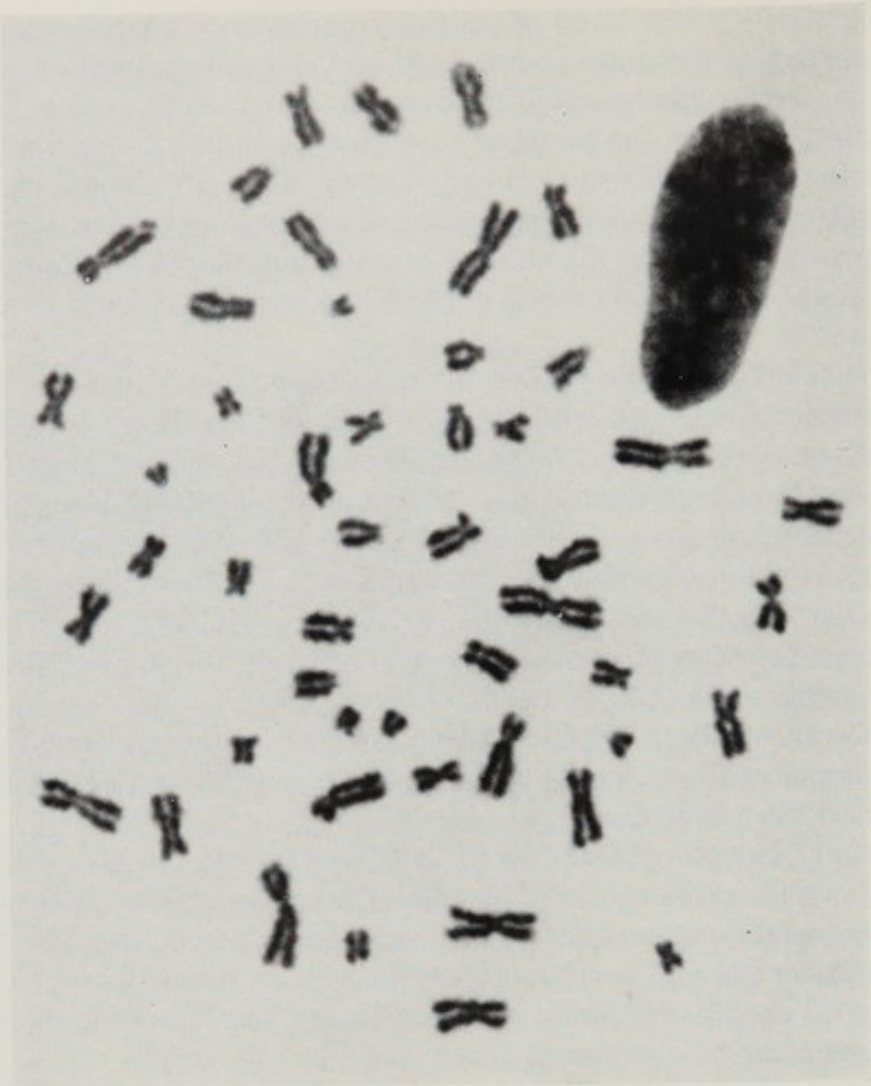


FIG. 10.8. Cell with 49 chromosomes. XXXXY gonadal dysgenesis.

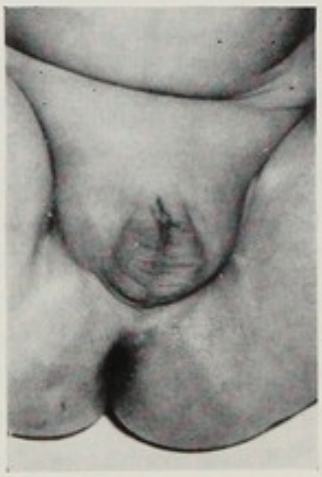


FIG. 10.8a. XXXXY infant of 3½ months. Penis of less than 1 cm in length buried in the interscrotal fissure. Sacs with pseudo-labial features.

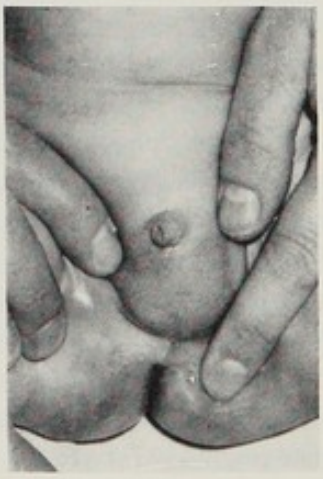


FIG. 10.8b. XXXXY infant of 3½ months. Penis of less than 1 cm in length buried in the interscrotal fissure. Sacs with pseudo-labial features.

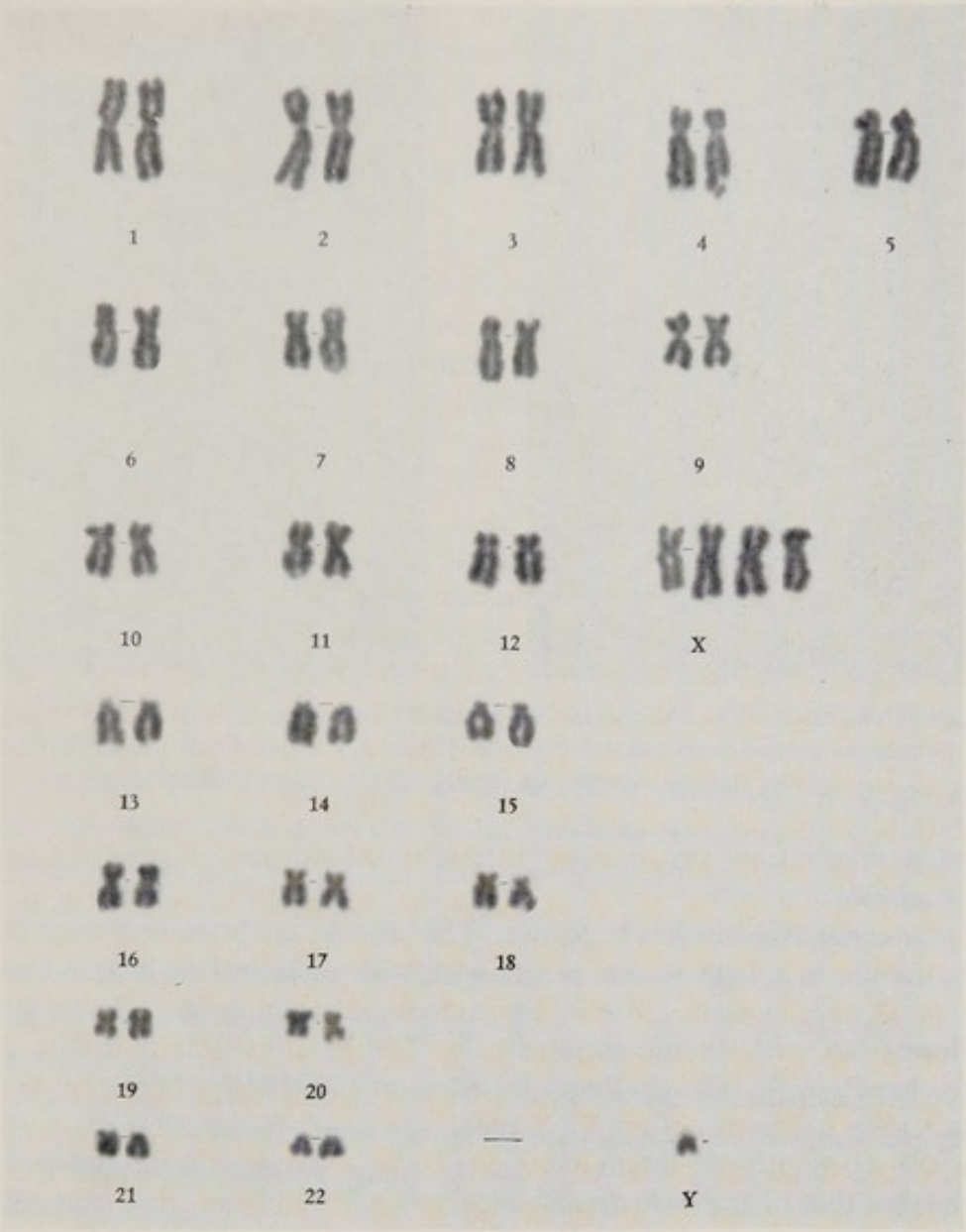


FIG. 10.9. Karyotype with 49 chromosomes. XXXXY gonadal dysgenesis.

thymidine may be expected to yield additional information (Mukherjee *et al.*, 1963b; Rowley *et al.*, 1963) (p. 281). The ages of the subjects range from 3½ months to 24 years. The birth weight at term is sometimes less than normal. The karyotypes of the parents and sibs wherever they were studied were normal. These families were not distinguished by excessive number of abortions. The average age of the mothers at birth of the XXXXY child is 28 years 10 months, fathers 31 years 2 months.

(1) Mental deficiency is constant, reflected from the first months of life in delay in mental awakening (Turpin *et al.*). It was accompanied in 6 out of 15 by microcephalia: the IQ varied from 22 to 66. The EEG may be abnormal with

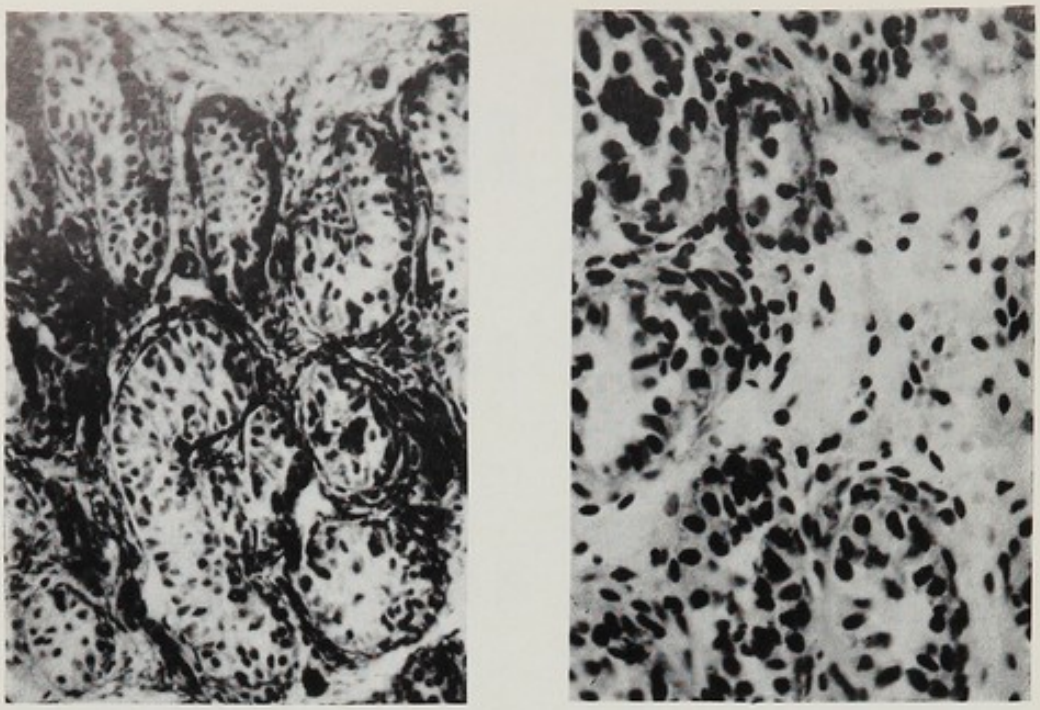


FIG. 10.9a and 10.9b. Testicular preparations from previous XXXXY infant. Numerous seminiferous tubules of normal size and without cell differentiation, in a loose stroma. Absence of Leydig cells in the interstitital tissue.

retarded maturation and convulsions. One infant was affected by internal hydrocephaly.

(2) Gonadal dysgenesis is important. The testicles are often very small, sometimes minute in a high scrotal position or with unilateral or bilateral ectopy. In general, they contain more or less numerous seminiferous tubules without hyalinosis but with undifferentiated cells. The germinal line is totally absent as are Leydig cells. The cytological aspects of the testicles of the 19-year-old subject were suggestive of an XXY type.

In the 4½-month-old infant mentioned above urinary hormonal excretion apart from that of the oestrogens which was a little higher than expected, did not diverge from normal. Overall and fractionated study of the 17-ketosteroids of the 24 hr urine of a 12-year-old subject (Fraccaro) gave results resembling those of older subjects of both sexes or of castrated males.

The urinary gonadotrophins of an adult were increased (Miller).

Penis, scrotum and prostate are small but distinct. There is no hypospadias. The genital apparatus despite this hypotrophy is typically masculine without Müllerian residues. Attention has been drawn to the eunuchoid morphology of the subjects aged 12, 21 and 24 years.

(3) More or less frequent skeletal anomalies are often noted and offer interesting analogies between the cases: microcephalia (6 cases); brachycephalia with or without microcephalia (5 cases); abnormally large glabella and underdeveloped frontal sinuses; prognathism; partial fusion of vertebral bodies; extra vertebrae; epiphyseal anomalies (metacarpal pseudo-epiphyses); anomalies of the middle joint of the little finger; coxa valga; radio-ulnar malformations

(proximal hypertrophy of the ulna); more or less complete proximal radio-ulnar synostosis (10 in 15 cases); sometimes retarded osseous maturation (8 of 12 cases) and frequent muscular hypotonia in infants.

(4) The fingerprints of these subjects are distinguished in certain cases by too numerous arches (Fraccaro *et al.*) which cannot be considered constant (Turpin *et al.*; Lamy *et al.*; Prader *et al.*).

Moreover, XXXXY subjects may be affected by malformations common to numerous congenital disorders: webbed neck, clinodactyly of the fifth finger, cleft palate, pyelo-ureteral dilatations, heart conditions, strabismus, myopia, hypertelorism and epicanthus. An example appears in a series already burdened with familial anomalies: lymphoid leukaemia in the father of the XXXXY subject, trisomy 21 in a paternal aunt, trisomy 21 in the son of another paternal aunt herself of normal karyotype (Miller *et al.*). The karyotypes of these two cases were free 21 trisomies.

In another case (Thuline *et al.*) an XXXXY subject suffered from dyschromatopsia found in his mother, brother and maternal grandmother.

Five families have been studied by the Xg group criterion and in each case the same group was found among the XXXXY subjects and their parents: either Xg (a−) (Barr *et al.*) or Xg (a+) (Barr *et al.*; Day *et al.*; Atkins and Connelly, Prader *et al.*).

The XXXXY type is a variety of Klinefelter's syndrome. It seems more serious, in particular, because of the mental deficiency and malformations which accompany it than the XXY type. Supplementary observations are necessary to define the differences between them.

XXXYY

Among the theoretical consequences of an iterative non-disjunction during spermatogenesis, non-disjunction during the first, then the second meiotic mitosis is the appearance of XXYY gametes and later, the possibility of XXXYY zygotes.

This zygotic type may also result from a mitotic anomaly during the first cleavage of an XXY zygote with a selective advantage of the XXXYY over the X0.

This XXXYY variety was observed (Josephine and Brown, 1962) in a mentally defective adult with "variant" of Klinefelter's syndrome. This eunuchoid subject with gynaecomastia was 1·80 m in height and also distinguished by signs of acromegalia. Gonadal biopsy revealed only a testicular stroma. This observation (Gray and Josephine, 1963) raises the problem of the relations between two Y chromosomes and the skeletal anomalies.

II. Forms with mosaicism

1. Variety X0/XY

The diversity of phenotypic expression of this mosaic leads one to distinguish four groups. This distinction, because of its uncertainties, is only a tentative measure taking karyotypes very much into account.

(a) Hermaphroditism is sometimes considered to be the most probable diagnosis (p. 231).

(b) Masculine pseudo-hermaphroditism is occasionally the diagnosis adopted and it has been applied to possible variants of testicular feminization (p. 245).

(c) Turner's syndrome is suggested by other observations. Two examples have been published (Jacobs *et al.*, 1961c). The external genitals of these females were hypoplastic but normal. Laparotomy revealed very rudimentary gonads, on the one side, connective tissue with accumulation of cells recalling thecal cells and on the other, an ovarian stroma without follicles. In neither case was there any sign of testicular tissue. Chromatin was negative. The X0/XY mosaic was predominantly XY in the blood, X0 in the skin cells.

While these two cases may be likened to Turner's syndrome, others are more difficult to classify. This applies to the case of Blank *et al.* (1960) of a 55-year-old female with genital and mammary hypoplasia and intelligence less than average. Only one gonad was found. She had a possible ovary on the left but it could not be examined. Blood sampling revealed XY cells and others in equivalent number presumed to be X0.

The same remarks apply to the observation of Judge *et al.* (1962) of an X0/XY tall subject of high intelligence and female phenotypic orientation but with primary amenorrhoea, sparse axillary hair and undeveloped breasts. The right gonad was made up of an ovarian stroma without primary follicles; the left gonad was not studied.

(d) X0/XY of masculine phenotype is another possibility. It has been reported by de la Chapelle *et al.* (1962c). Their observation concerns a chromatin-negative subject of X0/XY karyotype with hypospadias and moderate feminization of the breasts. Examination of the left scrotal testicle revealed absence of spermatogenesis, thickening of the tubular membranes, atypical pattern of Sertoli cells and hyperplasia of the Leydig cells. Scrotal examination on the right revealed no gonads. Excretion of gonadotrophins in 24 hr exceeded 40 mouse units. In the absence of supplementary information which only laparotomy could provide, it is impossible to draw any conclusions from this case.

The study by Ferrier *et al.* (1963) of a 35-year-old male of normal habitus was justified by the very considerable hypotrophy of the right testicle (orchidopexia at 5 years) and a little less of the left testicle. On this side a testicular biopsy revealed the absence of spermatogenesis with hyalinization of the tubular membranes and presence of Sertoli and many Leydig cells. The level of gonadotrophins was more than ten times higher than average; the cell nuclei had no sex-chromatin mass. Of 50 cells (blood) 25 XY with 46 chromosomes and

22 X0 with 45 were counted. In the absence of right testicular biopsy it was impossible to dismiss, as the authors note, the diagnosis of hermaphroditism which the phenotype made unlikely.

It is useful to compare these two observations with that of Lewis *et al.* (1963d) of a married subject of masculine type without children, operated on at first for a left malignant seminoma growing on a cryptorchid testicle. But in this hypospadic subject with vulviform scrotum, a perineal orifice led through a rudimentary vagina to a uterus. Sixteen years after the intervention for seminoma uterine cancer justified a further operation. A rudimentary right Fallopian tube was found and in ovarian position a small mass not of ovarian tissue. Karyotypic analysis (blood) revealed in 30 cells: 17 X0 and 9 XY.

The diversity of expression of X0/XY mosaicism is not surprising. Development of each primary gonad depends on the relative proportion in it of X0 and XY cells. Numerous varieties of gonadal dimorphism correspond to multiple cell combinations. An extreme variety involving a testicle on one side (XY) and ovarian dysgenesis on the other (X0) is conceivable. It would be equivalent in a single ambiguous individual to two heterokaryotic monozygotes, one XY, the other X0, a first example of which (Turpin *et al.*, 1961e) has been confirmed (Chapter 13).

With increase in the number of cytogenetic checks in different regions the number of unrecognized X0/XY intersexualities must shrink. But this evidence will only be valid if supplemented by cytological examination of the gonad.

The observations of sex ambiguity if a mosaicism is not systematically investigated and if the gonads are not systematically studied are likely to be improperly interpreted.

(2) XX/XXY variety

When they verified the constitution of the XXY variety of the Klinefelter's syndrome, Ford *et al.* (1959g) found from examination of the bone marrow in 65 cells 44 with 47 and 13 with 46 chromosomes. Analysis of the first cells indicated an XXY formula; 11 of the second an XX formula. The anatomical features of the gonad of this patient are not mentioned. After eliminating the hypothesis of an artefact, Ford assumed the possibility of a genuine XX/XXY mosaic in the bone marrow, the first example of human chromosome mosaicism. He suggested the loss of a Y chromosome through mitotic non-disjunction during the first cleavages of an XXY zygote. This suggestion of the loss of a "lagging" Y may draw support from the example of the above-mentioned XY–X0 heterokaryotic monozygotism.

A second example (Crooke and Hayward, 1960; Hayward, 1960a) of an XX/XXY mosaicism was reported shortly afterwards in a 37-year-old male whose psychopathy was accompanied by signs suggestive of Klinefelter's syndrome. Gonadal histology is not mentioned. From examination of the bone marrow an XX/XXY constitution was found. Jugal chromatin was positive, but, a point which the observation does not explain, is that of 250 chromatin-positive cells 83 per cent contained one and the remainder two sex-chromatin masses.

Since then this phenotype has been found (Turpin *et al.*, 1962c) in a herma-

phrodite who thus furnishes an example of concordance between anatomo-clinical and gonosomal ambosexuality (p. 234).

(3) XX/XY variety

This variety was found in a 21-year-old subject of feminine phenotype but primary amenorrhoeic (Forteza *et al.*, 1963c). The only anomaly of development detected by clinical examination was slight hypoplasia of the external genitals. Laparotomy was performed showing a rudimentary uterus, hypoplastic and elongated Fallopian tubes without pavillion, gonads set directly on the broad ligament and whose length did not exceed on the left 8 mm and on the right, 1 cm. Histological examination of one of them revealed under the very thick albuginea only a stroma of moderate cell density and without ovular formations. The karyotype established on a direct preparation of bone marrow revealed of 30 cells 27 with 46 chromosomes including 14 XX and 13 XY. The chromatin studied in buccal and vaginal cells was masculine and 500 leukocytes were devoid of drumsticks. The authors propose the diagnosis of "pure gonadal dysgenesis" through a "Jost" effect. This observation raises several problems. On the one hand, that of the chromosomal type of this pure gonadal dysgenesis (Swyer's syndrome, 1955) previously reported in XX subjects (p. 172) and XY (p. 173); on the other hand, that of the consequences of the XX/XY mosaicism so far observed in an intersex "true hermaphrodite" (p. 232).

These questions cannot be answered in the absence of more extensive karyotypic study, in particular, of the skin and gonads. The relation often found between the buccal chromatin type and the skin chromosomal type (p. 281) suggests that the latter is here perhaps XY.

(4) X0/XYY variety

This variety of mosaicism involving the Y chromosome concerns two subjects of feminine phenotype.

(a) The first case (Jacobs *et al.*, 1961c) was that of a 20-year-old female of short stature, primary amenorrhoeic with webbed neck, hyperplasia of the external genitals and chromatin negative. In the absence of laparotomy the anatomical features of the gonads could not be defined. Chromosomal analysis by skin sampling indicated in 61 cells 60 of the X0 type and 1 of the XYY type. Analysis by blood sampling indicated in 99 cells, 69 X0, 27 XYY and 3XY. Although the possibility of an X0/XY/XYY mosaic could not be excluded, the authors concluded that the X0/XYY variety was probable. It would be interesting to know if with this majority of X0 cells this subject belonged by gonadal dysgenesis to the Turner's syndrome.

(b) The second case (Cooper *et al.*, 1962b) was that of a 16-year-old intelligent girl with gonadal dysgenesis. An X0/XYY mosaicism was revealed by two karyotypic examinations: one blood which showed only XYY cells, the other skin which revealed in 60 cells 45 X0 and 2 XYY.

The cell distributions of these two examples of mosaicism are not comparable. The blood culture showed in one XYY cells and in the other, a mixture with predominance of X0 (69) over XYY (27); the skin culture, on the other hand,

gave very comparable results with predominance of X0 over XYY, 60 against 1, in one case, 45 against 2 in the other. These distributions may each reflect a type of cell architecture in the initial stage of embryogenesis or preferential selection by regions or be the result of both mechanisms.

(5) Other varieties

(a) Skin biopsy examination (S. Bergman *et al.*, 1960; Nowakowski *et al.*, 1960) of 13 subjects with Klinefelter's syndrome revealed in some a complex XX/XXY, XY/XXY and X0/XXY mosaic. In one of the cases, 20 per cent of the cells had 48 chromosomes due to an extra one similar to a 16 in addition to the XXY formula. The interpretation of these interesting observations requires additional information.

(b) An inquiry (Maclean *et al.*, 1961) recently completed (Court Brown *et al.*, 1964) after revealing 21 chromatin-positive in 10,725 newborn male infants was followed by karyotypic examination of these children. Sixteen subjects were studied and 10 were XXY, 5 XY/XXY and one XXYY.

Subjects with this XY/XXY mosaic are said in general to have an appearance differing little from that of the patient with Klinefelter's syndrome (Sandberg *et al.*, 1960b; Klotz *et al.*, 1962b). However, a 16-year-old boy had a normal appearance and intelligence higher than average (Baikie *et al.*, 1961a). These patients may be fertile (Court Brown *et al.*, 1964).

They appear to be affected by leukaemia or neoplastic processes more often than due to coincidence.

During karyotypic study of 18 cases of chronic myeloid leukaemia (Toughe *et al.*, 1961) chromosome Ph^1 was found in 13 of these patients; one of the latter in addition had an XY/XXY formula and clinical signs of Klinefelter's syndrome.

Sometimes, the Ph^1 chromosome is found without a superimposed leukaemia. It was encountered in a mentally defective chromatin-negative hypospadic and cryptorchid subject with skeletal anomalies and examination of the blood revealed a probable XY/XXY mosaicism and, in addition, both in the cells with 46 and 47 chromosomes a deletion of one of the small acrocentrics (22?). Stadler *et al.* (1963) who quote this observation of Pfeiffer *et al.* (1962e) do not speak of a superimposed leukaemic process.

Another observation (Lubs, Jr., 1962) concerns an XY/XXY subject with Klinefelter's syndrome complicated by an "oat cell" carcinoma of the lung.

As for the boy studied by Baikie *et al.* (1961a) he had two brothers who succumbed to acute lymphatic leukaemia in one case, and myeloblastic in the other.

(c) A karyotypic study was made (Barr *et al.*, 1962b) in a mental defective 31-year-old male (IQ 32, Wechsler) with as outstanding symptoms a significant proportion of buccal, skin and Leydig cells with one or two sex-chromatin masses. The blood and bone marrow cultures gave only an XY formula whereas those of the skin and a testicle had formulae interpreted as XY/XXXY. It is interesting to note, as do the authors, that the normal results of urinary hormone and testicular biopsy checks discounted diagnosis of Klinefelter's syndrome and that this subject was clinically normal apart from the mental deficiency.

On the other hand, it is worth noting a connection between the normal karyotype obtained by blood culture and the virtual absence of a chromatin appendix in the leukocytes (1 per 2800).

(d) Several observations of gonado-somatic dysgenesis in mentally defective persons are examples of XXXXY constitution with mosaicism.

The first (Harnden and Jacobs, 1961c; Maclean *et al.*, 1962b) was an XXXY/XXXXY mosaic, with 104 cells with 48 chromosomes and 42 with 49 out of a total of 149. It was found in a eunuchoid 39-year-old mentally defective male with normal penis but scrotal testicle presumed to be hypoplastic. He was chromatin-positive with nuclei with one, two or three sex-chromatin masses.

A second very comparable case (Court Brown *et al.*, 1964) concerns an oligophrenic 27-year-old with normal penis but testicular and scrotal hypoplasia and signs suggestive of radio-ulnar synostosis. At birth his mother was 30 and his father 28 years old. Karyotypic study revealed a XXXY/XXXXY mosaicism although a supplementary XXY was possible (blood and skin). Two other cases of mosaicism of the XXXXY type (p. 204) but with XXXXY predominant (Anders *et al.*, 1961; Prader *et al.*, 1964) have been described.

A mosaicism XXXY/XXXXY/XXXXYY with some XXXYY and XXYY cells was found from blood culture of a 28-year-old mentally defective male, of gynoid form, hypogonadic with adiposity and gynaecomastia (Gilbert-Dreyfus *et al.*, 1963).

(e) A male subject with clinical signs resembling Turner's syndrome but intra-abdominal testicles at first considered as an example of inversion of the sexual phenotype (Oikawa and Blizzard, 1961) since the karyotype established by bone marrow culture was XX, appeared subsequently to be a chromosomal variant of Klinefelter's syndrome (Solomon *et al.*, 1963).

Further chromosomal analysis (testicle and blood) showed indeed a mosaic in which 32 of 94 cells had 46 chromosomes and 51 had 47. The latter possessed an X chromosome, a large one considered as a long-armed X-isochromosome and a small chromosome suggestive of a 19–20. It is therefore assumed that the latter contained Y elements. The cells with 46 chromosomes differed from the preceding ones in absence of this small chromosome. Most of the paternal cells had 46 chromosomes (35/36) but of 9 karyotypes 5 had a Y and 4, instead of the Y, possessed a chromosome resembling a 16–18.

The authors assume that fertilization with an abnormal Y chromosome followed by a new combination giving anomalies in two of three sex chromosomes was the origin of this curious observation. The mother and sister of this subject had a normal karyotype.

Other observations of gonosomal mosaicism, according to the interpretations of their authors, come under the headings hermaphroditism and pseudo-hermaphroditism.

CHAPTER II

Modifications of Gonosomal Structure

VARIOUS anomalies of the sexual phenotype have been linked with structural aberrations of the X or Y chromosome.

ABERRATIONS IN STRUCTURE OF THE X CHROMOSOME

This study gives examples, in the heterozygous state, of an X chromosome of abnormal size through depletion or addition.

The mechanisms usually invoked are deficiency or duplication.

Deficiency, in fact, may account for reduction in the size of the long arm or the short arm of the X chromosome. Deficiency of the long arm, according to the examples reported, concerns only part of this arm: $\mathbf{X}^{ld}$ (deletion of the long arm), that of the short arm is almost total: $\mathbf{X}^{cd}$ (deletion of the short or "curt" arm).

The deficiency may be either intercalary or terminal. In the first case (deletion) it is thought to require two ruptures which permit secondary fusion of the telomere; in the second, it is thought to require only one rupture, an accident exemplified in *Zea maïs*.

Duplication may explain an abnormally long X or abnormally short one, depending on whether it produces a neochromosome consisting of two long arms or two short arms of the X chromosome. This duplication from the theoretical point of view presupposes either a rupture within the centromere for one of the X chromosomes and outside it for the other, that is, maldivision (transverse division) of the centromere common to the two chromatids. This maldivision is thought to be followed by formation of a "long-armed isochromosome of X", $\mathbf{X}^{li}$, and a "curt-arm isochromosome" $\mathbf{X}^{ci}$. These isochromosomes are thought to be the human equivalents of anomalies so far observed only in plants (Håkansson, 1933; Darlington, 1939, 1940).

Other aberrations have been described: "round ring X", $\mathbf{X}^{r}$; others have been reported—presumptive "X fragments" $\mathbf{X}^{f}$. The identification of these neochromosomes may be assisted by the concordant variations of the chromatin mass and by incorporation of ^{3}H-thymidine. These structural aberrations may be found either as mosaics or not and mostly in the female.

Aberrations in the female

I. Varieties without mosaicism

(a) XX^{1d} type

The first example of $X\mathbf{X}^{1d}$ aberration, probable partial deletion of the long arm of X, was observed in 1960 by Jacobs *et al.* (1960a) in a 37-year-old female of below normal intelligence. Married for 11 years, she came from a family without any notable history of pathology. Of medium height, about 1·55 m, she claimed attention by the infantile character of her external genital organs, mammary agenesis and paucity of pubic and axillary hair. Under the influence of oestrogens some menstruations had appeared. Laparotomy revealed hypoplasia of the uterus and gonads; they were formed of rudimentary ovarian tissue without trace of testicular tissue and with few primary follicles on only one side.

Six cultures (bone marrow, blood, skin [2], gonads) gave 46 chromosomes but the karyotype analysed in 34 cells diverged from normal in the absence of an X and the presence of an extra element similar to a chromosome 16. The buccal chromatin was weakly positive with smaller than normal masses present in 7 per cent of the cells and absence of drumsticks.

After discounting for anatomo-clinical reasons the coincidence of Turner's syndrome and trisomy 16 or the possibility of a translocation X ~ Y the authors suggested a loss of substance of a long arm of one of the X chromosomes and hence, the formula $X\mathbf{X}^{1d}$.

The small size of the chromatin body and its lower DNA content have since been reported in cases of presumed X chromosome deletion (Lindsten, 1963a).

An observation similar from the anatomo-clinical and karyotypic point of view (Grouchy *et al.*, 1961e) of partial deletion of the long arm of an X chromosome showed about 15 per cent chromatin-positive buccal cells with masses of normal dimensions. An ovarian cuneiform resection did not lead to menstruation. The authors interpreted this case as a severe form of Stein-Leventhal syndrome.

(b) XX^{cd} type

A second variety of X chromosome deletion (Jacobs *et al.*, 1961c) was observed in a primary amenorrhoeic woman. This variety was considered as a deletion of most of the short arm of X. Thus, the gonosomal formula consisted of a normal X and a reduced X similar in size to a large acrocentric but differing from chromosomes 13 and 14 in the absence of a satellite. The buccal chromatin was positive in 30 per cent but the masses were smaller than normal. In 500 polynuclear cells, 18 drumsticks and 3 sessile appendages were found.

It is probable that these examples of loss of substance of part of the large arm of the X chromosome or the greater part of the small arm are compatible with life since they are heterozygotes.

Heterozygous deficiency of half of the X chromosome is lethal in female *D. melanogaster*.

(c) XX^r type

The $\mathbf{X^r}$, or round or ring-shaped X chromosome, has been described in a female with gonado-somatic dysgenesis (Lindsten and Tillinger, 1962c), its karyotypic formula corresponding to the scheme $XO/X\mathbf{X^r}/X\mathbf{X^rX^r}$ (see below).

Since then another probable case was found by blood and skin cultures of a subject with Turner's syndrome (Hustinx, 1963b). The Barr body existed in some cells of skin origin. The hypothesis of a peripheral localization of this $\mathbf{X^r}$ in the metaphase figures had been suggested but it was not verified.

To explain the formation of this atypical X the fusion of its two ends both reduced by a deletion, has been suggested.

(d) XX^{11} type

The variety $X\mathbf{X^{11}}$, probable long-armed isochromosome, was detected by Fraccaro *et al.* (1960d) in three primary amenorrhoeic females with Turner's syndrome: small stature, short neck, infantile external genitals, raised gonadotrophins and reduced urinary oestrogens. Uterine hypoplasia and rudimentary ovaries were verified in two of these females at laparotomy.

A fourth case (Jacobs *et al.*, 1961c) showed similar features to the first three. Gonadal inspection at laparotomy could not be made. In these four subjects the chromosome modal number was 46 (skin and bone marrow for the first three cases; skin and blood for the fourth). But the karyotype diverged from normal in the absence of an X and in the presence of a large extra chromosome similar to chromosome 3.

The four women were chromatin positive with no other special features for the first three. In the fourth the proportion of positive buccal cells was 60 per cent and about 30 per cent of these had abnormally large masses. In addition, 20 per cent polynuclears with drumsticks were found, for the most part of large dimensions.

Thus, a common karyotypic anomaly unites these four observations; presence of an extra chromosome similar to chromosome 3 and absence of a chromosome in group 6–12 X.

The hypothesis of trisomy 3 appeared very unlikely on the strength of the evidence then available.

The hypothesis of a duplication of the short arm of an X chromosome or translocation of an autosomal segment onto this short arm was not accepted. It appeared improbable that a modification of this type could have produced in four different subjects the same neochromosome and the same anatomo-clinical signs.

The hypothesis of neochromosome X formed by the two long arms of two homologous X chromosomes was considered as the most plausible. Accordingly, these females were considered as examples of short-arm monosomy and long-arm trisomy. Two of these three long arms are therefore homologues.

This interpretation had the advantage of explaining the presence of an abnormally large sex-chromatin mass in one of the patients.

It has since been checked by the ^{3}H-thymidine technique (Muldal *et al.*, 1963;

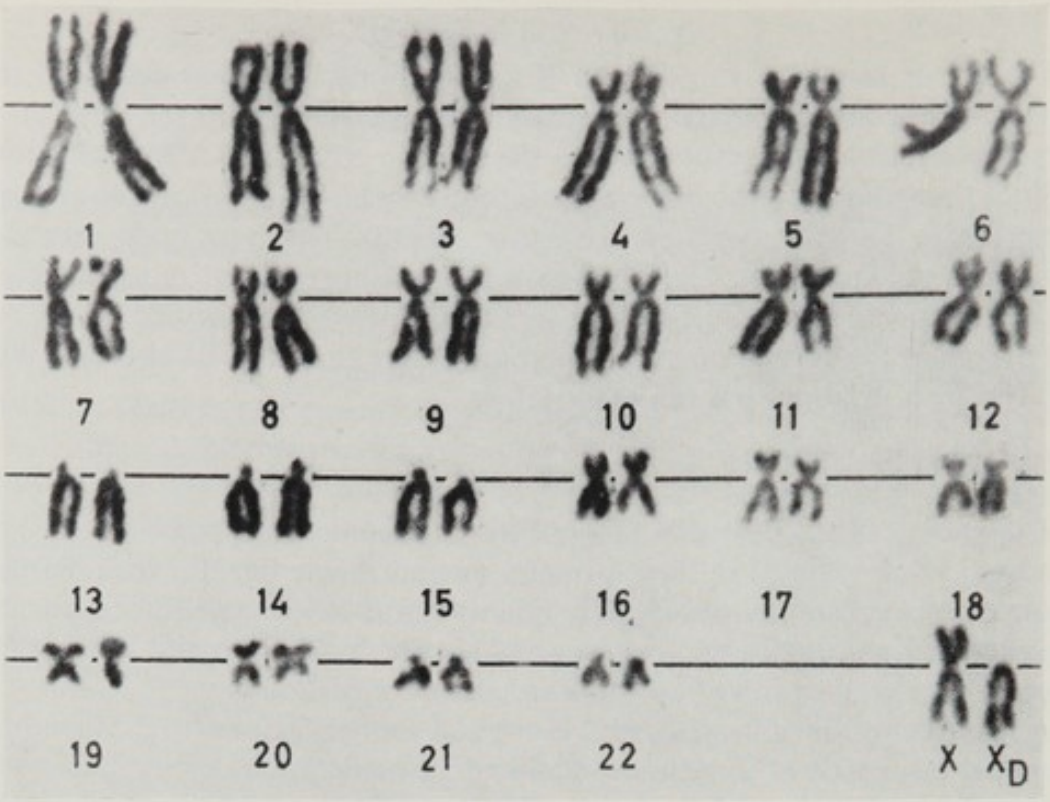


FIG. 11.1. Karyotype showing deletion of the short arm of an X chromosome. Type XX^{cd}. (Photograph by courtesy of Dr. Lindsten.)

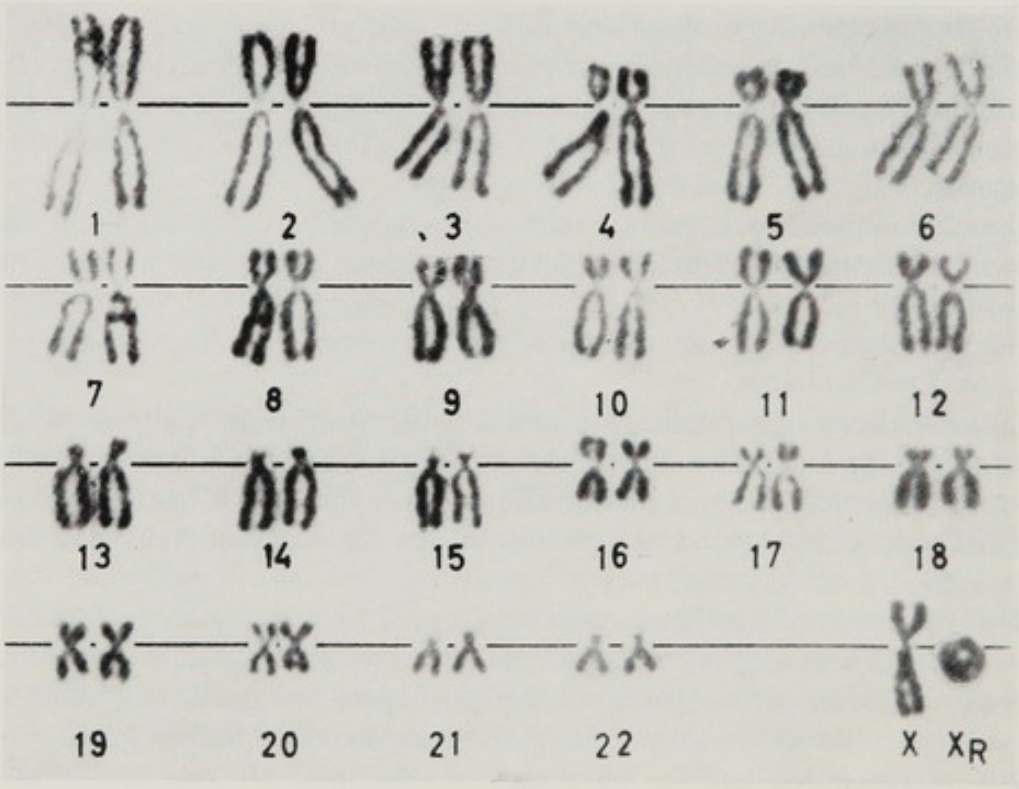


FIG. 11.2. Karyotype showing a ring chromosome X. Type XX^r. (Photograph by courtesy of Dr. Lindsten.)

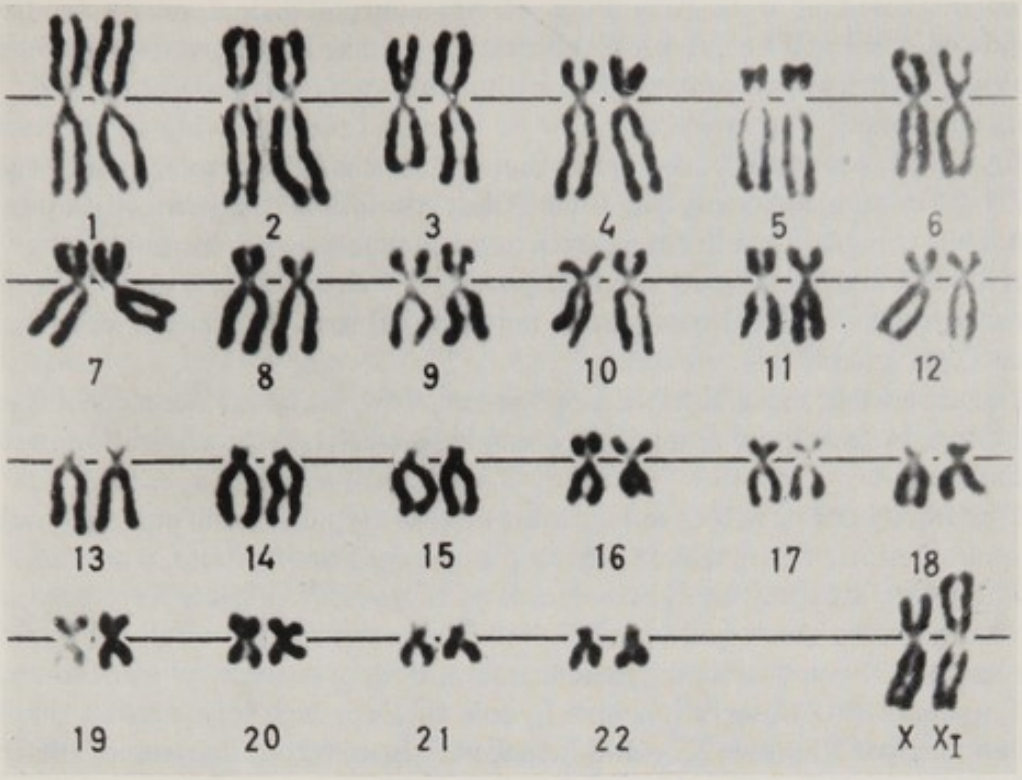


FIG. 11.3. Karyotype showing an X isochromosome. Type XX^{1i}. (Photograph by courtesy of Dr. Lindsten.)

Taft and Brooks, 1963). From the time and course of incorporation of ^{3}H-thymidine this test demonstrated the similarity of behaviour of the X and the presumed iso-X chromosomes. It also demonstrated symmetry of incorporation in the two arms of the iso-X and, finally, participation of this iso-X chromosome as a whole in the formation of the Barr body.

This method applied to karyotypic analysis of other cases of gonosomal aberrations gave useful information on certain cytological details (p. 281).

Two other XX^{1i} cases (Sparkes, 1963) differed from the preceding ones in coincidence in two unrelated women with primary amenorrhoea of phenotypic patterns suggestive of Turner's syndrome and Hashimoto's disease.

Two others differed even more (Hamerton *et al.*, 1962b; Carr *et al.*, 1963) especially in the extent of the associated visceral malformations. The latter authors found at autopsy of an XX^{1i} chromatin-positive infant interventricular communication, foramen ovalis, absence of gall bladder, absence of right kidney, iliac left kidney, bicollis bicornis uterus and vaginal septum. In contrast, the ovaries and primary follicles were practically normal. After accepting, not without hesitation, the hypothesis of an XX^{1i} formula, the authors mention the information they have received on similar XX^{1i} cases: from one source, three renal anomalies in six subjects studied, all free of cardiovascular anomalies, and also a seventh (Polani, 1963) and from another source, a renal anomaly in four other patients (Lindsten *et al.*, 1963c).

The frequency of twins in families with an XX^{1i} or $X0/XX^{1i}$ female appears

to be increased as it is in families of X0 subjects with Turner's syndrome (Lindsten, 1963a). Thus, the $X\mathbf{X}^{ii}$ aberration may be expressed in a chromatin-positive Turner's syndrome with or without serious clinical deformations.

Its cytogenetic diagnosis may now be based on the following signs: level of DNA of the chromatin body higher than that for good controls; incorporation of ^{3}H-thymidine suggesting that for an X but continuing symmetrically along the two arms; length of both these arms approximately equal to that of the long arm of a normal X chromosome. The presence of drumsticks and an area of the chromatin masses sometimes greater than normal have been mentioned (Jacobs *et al.*, 1961c; Lindsten, 1963a).

The absence of mosaicism despite bone marrow, blood and skin checks, is an argument in favour of premeiotic or meiotic origin of the aberration in these patients.

The search for possible transmission of this atypical X chromosome seems by examination of parents and sibs so far to have been fruitless.

The results of study by ^{3}H-thymidine of these $X\mathbf{X}^{ii}$ subjects have been used to challenge the thesis of Mary F. Lyon. They showed that in all cells studied the inactive X responsible for the chromatin body was the isochromosome.

To square this observation and Lyon's thesis it has been argued that the cells—inactive X, active $\mathbf{X}^{ii}$—and hence, with hemizygous deletion of the short arm of X, are lethal (Gartler and Sparkes, 1963).

These subjects, like X0 subjects (p. 285) are said to be stunted from the start of embryogenesis by the loss of about half their cells retaining only those in which the active is the normal X. This explanation runs into the difficulties outlined elsewhere (p. 285).

(e) Other varieties

(i) In its karyotypic similarities, the observation of Edwards (1961a) of a female distinguished clinically only by primary amenorrhoea comes close to the preceding type. Buccal and vaginal chromatin was positive, with more numerous and larger masses than normal. Some buccal masses (5 per cent) were bipartite, 13 neutrophils in 1100 carried a drumstick.

A karyotype with 46 chromosomes was found by fibroblast culture, differing from normal in the absence of an X chromosome and the presence of an atypical chromosome resembling an X the long arm of which would have been approximately doubled. After discussion, the author accepts as the most likely hypothesis a duplication of the long arm of X although the possibility of translocation between heterologous chromosomes could not be dismissed. With this qualification, the probability of long-arm trisomy and short-arm disomy of the X chromosome appears the most plausible.

(ii) Another variety (Engel and Forbes, 1961) is based, like the preceding one, on a single observation and does not lend itself to a simple interpretation either. It was detected in a primary amenorrhoeic female of below normal intelligence. At the age of 14 years she had drawn attention to herself by obesity, height of 1·27 m and weight of 37·4 kg. Her external genitals were normal, but the uterus and ovaries were not found by rectal exploration. Pubic hair was sparse,

axillary hair absent and the breasts were not developed. These signs were accompanied by increased urinary gonadotrophins, positive chromatin in 33 per cent of the jugal cells and abnormally large drumsticks in 46 of 238 polynuclear cells.

Peripheral blood culture showed a modal number of 46 chromosomes; but the karyotypes diverged from normal in the absence of an X and presence of an extra chromosome with the dimensions of an X but with median centromere. After dismissing the hypotheses least compatible with the chromatin, karyotypic and clinical signs, the authors opted either for the hypothesis of a transposition of a segment of the long arm of an X to the short arm or of fusion of two homologous long arms of an X each amputated from an equivalent short segment.

Finally, they proposed the latter interpretation, explaining the median site of the centromere and the length of the chromosome. It implies, according to the authors, greater anomalies of the genic material than those which result from mere long-arm to short-arm segmental transposition.

(iii) A possible $X\mathbf{X}^{ci}$ type was observed in a female of average stature, with primary amenorrhoea, chromatin positive and whose ovaries were normal on palpation. Apart from this presumed anomaly, the karyotype was normal (Polani, 1961e).

(iv) As for the rare variety with 46 chromosomes X + minute, and chromatin negative, its nature remains undetermined, since the minute fragment may represent the centromeric region of an X or Y chromosome.

Structural aberrations of the X chromosome may, in principle, result from mitotic or meiotic anomalies. In the first case, the anomaly will occur after formation of the zygote. X-irradiation of the zygote may bring this about. In the second case, it would be the consequence of disturbance in paternal or maternal meiosis.

Various arguments favouring the paternal origin, in particular, of $\mathbf{X}^{1i}$, have been marshalled (Lindsten, 1963a). While the two X chromosomes during female meiosis are isopycnotic and seem less exposed to aberrations, X and Y at male meiosis are heteropycnotic. Sometimes, they appear at diakinesis as univalents (Ford and Hamerton, 1956c). Plant genetics (Sears, 1952) provide some examples of univalents which form isochromosomes.

Following up these arguments the same author invokes a shortage of girls and a relative increase in boys among the sibs of $X\mathbf{X}^{1i}$ and X0 subjects, said to be additional proof of paternal, meiotic origin of these gonosomal constitutional anomalies. It must be added that an $X0/X\mathbf{X}^{1i}$ case (Lindsten, 1963a) (see below) suggests from the results of study of segregation of the genes of daltonism and of the blood group Xg that this patient had received the X from her father.

This paternal responsibility cannot at present be linked with the influence of age. The hypothesis of disturbed meiotic segregation of genetic origin, a fact noted in *D. melanogaster*, (Sandler *et al.*, 1959) may be considered.

II. Varieties with mosaicism

(a) XX/XX^{1d} type

An example of mosaicism—normal line plus line with probable deletion of most of the long arm of an X chromosome, type XX/X**X**^{1d}—was found in a 22-year-old married woman with Stein-Leventhal syndrome (Grouchy *et al.*, 1961e). This diagnosis was justified by the features of the small ovaries surrounded by a whitish, pearly, smooth and hard shell. Histological examination revealed some small cystic follicles, others with thecal-cell hypertrophy and the absence of corpora lutea or albicans. The uterus was hypoplastic, the external genitals normal. In other respects the body of this female was normal, but since her menarche at 13·5 years she had menstruated only 6 times; urinary gonadotrophins were increased and the vaginal secretions hypoestrogenic. Cuneiform resection of the ovaries gave normal cycles. The chromatin was positive but aponeurotic culture revealed from analysis of 17 cells, 9 with normal karyotype and 8 with an X chromosome deprived of most of its long arm.

This observation suggests that the partial deletion of an X chromosome may be post-zygotic.

Comparing this example of partial deletion of the X chromosome with a similar case without mosaicism and with more marked Stein-Leventhal syndrome (Grouchy *et al.*, 1961e) the authors assumed that the normal cell line may have attenuated the effects of the abnormal line.

Another XX/X**X**^{1d} observation concerns a 21-year-old female of normal height but whose sexual infantilism was accompanied by primary amenorrhoea (Gropp *et al.*, 1963b). Buccal chromatin was positive but found in only 8·5 per cent of the cells.

(b) X0/XX^{1d} type

This type of mosaicism was detected in a subject of female phenotype (de la Chapelle, 1962b) whose mental retardation was complicated by gonado-somatic dysgenesis (horseshoe kidney). Blood culture pointed to a line apparently XO and another with 46 chromosomes but with an X chromosome reduced by partial deletion of the long arm. No drumsticks were found and the buccal chromatin masses were abnormally reduced in number and size.

(c) X0/XX^{cd} type

This karyotype with an X reduced by probable deletion of the short arm was found in a woman with signs of Turner's syndrome (Lindsten, 1963a). At 23 years she measured 1·37 m and a horseshoe kidney completed her dysgenesis.

(d) Types with X^{r}

The 22-year-old female who provides this example of a round or ring X (**X**^{r}) (Lindsten and Tillinger, 1962c) was affected by gonado-somatic dysgenesis, although the secondary sex characters were developed, with short stature (1·43 m), oligomenorrhoea, rarity of primary and Graafian follicles and double left kidney. Buccal chromatin was negative but positive in cultured cells. Drum-

sticks were found in certain leukocytes. While the skin karyotypic formula was X0, the more complex blood formula consisted mainly of two cell lines. One X0 gave 45 chromosomes; the other had 2 X with 46 chromosomes, one X of which was ring-shaped; a third line was represented by 3 XXX cells in 160 with 47, with two X chromosomes in the form of a ring. This formula corresponded to the scheme X0/X**X**^{r}/X**X**^{r} **X**^{r}.

This ring chromosome could not be the consequence of exposure to ionizing radiations. Its perennial pattern during several cell generations was verified. In size it was a chromosome 13–15. Its changes in line with the mitotic phases and the cell types with 46 or 47 chromosomes were analysed (Lindsten, 1963a). An additional chromosomal anomaly accompanied it: a 13–15 chromosome the short arm of which suggested a duplication, the distal segment being satellized. This abnormal 13–15 chromosome was found again in the father.

In all the X**X**^{r} cells the **X**^{r} chromosome alone behaved like a heteropycnotic X in the ^{3}H-thymidine test. This suggests that this ring chromosome resulted from end to end fusion of an X chromosome after the ends had been reduced by a distal deletion.

In certain features the following observation (Lüers *et al.*, 1963b) differs from the preceding one: 20-year-old amenorrhoeic female with infantile secondary sex characters, weakly positive buccal chromatin (9 per cent) and presence of some small sex chromatin masses with absence of drumsticks. Of 83 cells (2 cultures of leukocytes) 61 had 45 chromosomes X0, 1 had 45 with a ring X, 14 had 46 XX, 5 had 46 with ring X, 2 had 47 due to a small chromosomal fragment. This description falls within the scheme: X0/**X**^{r}0/XX/X**X**^{r}/XX**X**^{r}.

The variability of the dimensions of the ring X point to lability comparable with that of the ring chromosomes of maize: increased or reduced dimensions and possible disappearance.

The latter possibility, according to the authors, would explain the large number of X0 cells in their patient who had not been exposed to X-rays.

(e) ***X***^{f} *types*

The chromosomal fragment, presumed (**X**^{f}), noted in the above observation with **X**^{r} has also been reported in subjects with signs of Turner's syndrome 45/46 with X0/X**X**^{f} mosaicism (Ferrier *et al.*, 1962; de la Chapelle, 1962a).

It is pertinent to compare these findings with two observations of X0/X plus a minute chromosome in two subjects of female phenotype more or less suggestive of a Turner's syndrome and with primary amenorrhoea. The nature of this minute fragment, whether the centromeric region of an X or a Y remains uncertain (Court Brown *et al.*, 1964).

(f) ***X0/XX***^{i} *type*

An initial example was found in a 14½-year-old girl with clinical Turner's syndrome (Blank *et al.*, 1961). Examination of 130 cells from culture of two blood samples distinguished two lines: one of cells with 46 chromosomes having in the place of a normal X a long-armed X isochromosome, the other of cells with 45 chromosomes through absence of an X. The first line took in 90 per cent of

the 130 cells examined, the second 10 per cent. Nevertheless, 91 per cent of the buccal cells, that is, more than the normal average, were chromatin positive.

The interpretation proposed was that of a long-arm to short-arm transposition between the two chromatids of an X chromosome during the first zygotic cleavage. One of the blastomeres would then have received an X chromosome and the long-armed isochromosome, the other only an X, the short-armed isochromosome having been eliminated.

Added interest attached to this observation with the subsequent publication of similar cases (Lindsten, 1961, 1963; de la Chapelle, 1962a; Grouchy *et al.*, 1963e; Gropp *et al.*, 1963b).

These women have in common signs suggestive of Turner's syndrome: short stature, primary amenorrhoea, sometimes cubitus valgus, naevi, normal or abnormally elevated elimination of gonadotrophins and sometimes cardiovascular and renal anomalies (de la Chapelle). Coelioscopy showed gonadal aplasia in one of these women; in the others, vaginal palpation revealed very small gonads or did not detect them. For others, there is no mention of such exploration.

An X0/X**X**^{1i} patient of Lindsten was colour-blind (deutan group). Study in her family of the segregation of genes of the deutan form of daltonism and the Xg blood group suggested that the **X**^{1i} was of paternal origin and that the genes of this dyschromatopsia and of the Xg group were localized on the small arm of the X chromosome (Fraccaro and Lindsten, 1963). Another patient was affected, the only one in her family, by progressive cerebellar degeneration of hereditary type.

The X0/X**X**^{1i} mosaicism is accompanied in all cases by the presence of cells with single or even double sex chromatin masses. The proportion of chromatin-positive buccal cells varies: 91 per cent (Blank *et al.*), about 5 per cent (Grouchy *et al.*) and 7·5 per cent (Gropp *et al.*), which may be related to the karyotype of the skin cells. A relation has in fact been sometimes noted between the X0 skin formula and the chromatin-negative buccal cells and the XX formula and cells with one chromatin mass. The karyotypes of these two females were studied only in the blood.

The ^{3}H-thymidine technique and autoradiography have already found interesting applications in observations involving a presumed long-armed X isochromosome: X0/X**X**^{1i} (O.J. Miller *et al.*, 1962c; Muldal *et al.*, 1963; Lindsten *et al.*, 1963d; Gianelli, 1963). In an X0/X**X**^{1i} culture of leukocytes this isochromosome was always distinguished by the latest DNA replication (Mukherjee *et al.*, 1963a).

These observations may be linked with the X0/X**X**^{1i}/X**X**^{1i}**X**^{1i} female (Lindsten, 1963a), who again showed signs evocative of a Turner's syndrome.

(g) X0/XX/XX^{1i} *type*

The karyotypic examination of a primary amenorrhoeic female with mental retardation (Bompiani and Moneta, 1963b) disclosed this presumptive X0/XX/X**X**^{1i} karyotype with chromatin in 0·8 per cent (Klinger-Ludwig). Short neck, pigmented naevi and dyschromatopsia were accompanied by renal malformations, partially duplicated left renal pelvis and gonadal dysgenesis. The latter

was reflected in hypoplasia of the external genitalia, uterus and the ovaries. Examination of one of them showed that the cortical matter did not contain elements of the germinal series and that the medullary substance was formed of loose connective tissue, or vessels and tubular formations lined with a unicellular cubic epithelium.

Aberrations in the male

I. Varieties without mosaicism

Giant X, Y type

In a 15-year-old haemophilic boy with arachnodactyly and genital infantilism a neochromosome was found interpreted as a giant X. The jugal cells were chromatin negative, the karyotype (blood) with 46 chromosomes was distinguished by the absence of an X chromosome and the presence of a large chromosome of shape and size similar to chromosomes 1 or 2.

After discussion the authors (Elves and Israels, 1962b) liken this atypical chromosome to a giant X resulting from translocation between the two maternal X chromosomes during oogenesis. Examination of the mother who died 2 years earlier or of other members of the family could not be made. The interest of this observation lies in the possible relation between this rearrangement of an X and existence of an X-linked recessive disorder accompanied by anomalies of genital and skeletal development.

II. Varieties with mosaicism

(a) $X0/XX^{1d}Y$ type

This formula found in an infant (Miles *et al.*, 1962c) was accompanied by sexual ambiguity which justifies placing this observation in the category of pseudo-hermaphroditism (p. 246).

(b) $XXY/XX\mathbf{X}^{1d}Y$ type

This mosaic made up of two XXY clones, one distinguished by the additional presence of a probable $\mathbf{X}^{1d}$ neochromosome through partial deletion of the long arm, was accompanied (Crawfurd, 1961a) by signs of Klinefelter's syndrome in a subject who was additionally thalassaemic.

(c) $XX/X\mathbf{X}^{cd}$ type

This type of mosaicism was noted (Conen and Erkman, 1963b) in a newborn infant of ambiguous sex: hypospadias with bifid scrotum, right gonad appearing as a normal testicle and left gonad palpable in lateral inguinal ring, but with buccal positive chromatin. Blood culture revealed two cell lines in more or less equal proportion: one XX, the other $X\mathbf{X}^{cd}$.

STRUCTURAL ABERRATIONS OF THE Y CHROMOSOME

The remarks on the assessment, in part subjective, of certain X variations also apply to Y. The fact that its study in normal subjects so often gives contradictory results would appear to indicate the existence of considerable polymorphism.

According to some cytogeneticists, Y cannot be distinguished from the small acrocentric autosomes (Ford and Hamerton, 1956c), its size being the same (Levan and Hsu, 1959) and this opinion prevailed at Denver where it was considered to differ little from chromosomes 21 and 22.

In the view of others (Chu and Giles, 1959) and from the results of study among the Japanese population (Makino and Sasaki, 1961a) Y is the smallest of the acrocentrics of the G group.

In the view of others (Ferguson-Smith *et al.*, 1960d; Patau, 1960a; Hauschka *et al.*, 1962b) it is the largest. Others consider that its size is subject to great and even individual variations (Bender and Gooch, 1961a).

The general though not unanimous consensus of opinion is that the small arm of Y is usually smaller than those of the acrocentrics of the G group and that it is unsatellited (cf. Chapter 3).

The structural aberrations of the Y chromosome with or without mosaicism are anomalies through deficiency or excess.

The instances in which they are reported are often marked by discrepancies and whether it be a matter of the morphology of the Y chromosome or its functional value, the facts reported are too contradictory to lead to a definite conclusion. On the other hand, their possible relations to the structure of the Y chromosome must be borne in mind.

The Y chromosome is heterochromatic, if not altogether, at least in part. It is allocyclic from the point of view of spiralization and laying down of nucleic acids. Whenever this heterochromatic behaviour is variable and if the allocycly involves a certain irregularity, the strictness of a morphological comparison between Y and the small acrocentrics would become illusory.

On the other hand, heterochromatic regions appear in *Drosophila* if not devoid of genes, devoid at least of large genes. If, therefore, the structural variations of the Y chromosome involve only heterochromatic regions their phenotypic consequences may be negligible.

By analogy with the symbols proposed for X chromosome aberrations the possible interpretations of aberrations of the Y chromosome lead one to distinguish the deletion of part of the long arm of Y: $\mathbf{Y}^{1d}$; short (or curt) armed Y isochromosome: $\mathbf{Y}^{ci}$; giant Y or $\mathbf{Y}^{g}$.

I. Varieties without mosaicism

(1) Presumptive deletions of Y

In 1960, the case of a child $1\frac{1}{2}$ years old of male phenotype was reported (Turpin and Lejeune, 1960a). The leading features were sexual ambiguity with peniform clitoris and urogenital sinus which contrasted with the masculine type

of the intra-abdominal gonad. The testicles contained seminiferous tubules in Sertolian involution and without spermatogenic activity. A small retrovesical mass resembled a very hypoplastic uterus. This dysgenesis was complicated by dextrocardia. These developmental disturbances were accompanied by difficulties of pairing of the acrocentrics of the G group. After discussion, the interpretation proposed was that of a translocation Y ~ 21 with partial deletion of the Y chromosome, an interpretation having no other value than that of a working hypothesis. This observation 44A + XY^{1d} suggested, in fact, that certain forms of male pseudo-hermaphroditism may be related to a deletion of the Y chromosome.

Subsequently, observations of a more extensive deletion were reported.

Vaharu *et al.* (1961) reported on a subject of 4·5 years of female habitus. The mental retardation was accompanied by delay in physical development, some signs of hirsutism, minor malformations of the face, internal strabismus with neuro-motor disturbances and trembling, unnatural gait and clumsy grasp. In addition, an enlarged clitoris was noted with partial posterior fusion of the labia majora. The features of the gonads were not stated. Negative chromatin was accompanied by a karyotype with 45 chromosomes with a small chromosomal fragment. This fragment devoid of centromere was considered as a part of the Y chromosome (Y^f).

Another report (Muldal and Ockey, 1961c) describes the examination of five male subjects of the same family with "muscular dystrophy" transmitted as autosomal or X-linked dominant. Three of these five subjects including the propositus had hypospadias. Examination of the blood of four of them revealed a deletion of one-third to a half of the long arm of the Y chromosome; Y^{1d}. The authors considered the hypospadias as a minor intersex form.

However, a hypospadias is not reported in the observation on a 45-year-old sterile male (Van Wijck *et al.*, 1962) whose pronounced hypospermatogenesis was accompanied by incipient hyalinization of the seminiferous tubules. The Y was of reduced size.

Rupture of Y into two fragments, one translocated onto one of the large 13–15 acrocentrics, the other onto a small 21–22 chromosome is suggested (Lamy *et al.*, 1962) to explain the paradoxical 44A + XX appearance of a probable 44A + XXY karyotype. Perineal hypospadias with chromatin-positive Klinefelter's syndrome has been found, in fact, in a 20-month-old child. Histological study of the two gonads appeared to be concordant: testicular gland without embryonic remnants, thickened tubular vitreous without hyalinosis and normal Sertolian cells. No element could be interpreted as a gonocyte. These features differing from those of ectopic testicles could be considered as the first histological signs of Klinefelter's syndrome. The authors in view of the gravity of the anatomo-clinical syndrome refer to the possible role of a deletion of the Y chromosome simultaneous with its rupture.

A male subject (Oikawa and Blizzard, 1961) with mosaicism involving a presumptive chromosomal variant of an XXY formula comes under the heading of Klinefelter's syndrome (p. 214).

(2) Giant Y chromosomes

Difficulties of interpretation of examples of the "giant" Y chromosome are even greater. To show this we need only take the case (Bender and Gooch, 1961a) of an apparently normal Indo-European (Caucasian) whose somewhat heteropycnotic Y exceeded the normal size by some 15 per cent. In this connection the author stresses the variability of length of Y not only between normal individuals but also between cells of the same individual.

This Y of size greater than average is transmissible. It has been found in relatives in no wise differing from normal (A. Bishop *et al.*, 1962).

Sometimes, it is accompanied by a pathological state: sterility, oligospermia and moderate histological signs of hypospermatogenesis in a 35-year-old male. In size his Y chromosome was close to those of a large acrocentric, but the features of this chromosome in the relatives or father are not mentioned.

Finally, it is sometimes transmitted by a normal subject to his sons whether normal or not: normal father and his two sons of whom one had trisomy 21 (A. Bishop *et al.*, 1962); normal father and his deformed son (Makino *et al.*, 1963b); normal father and his two monozygotic twin sons one of whom was deformed (Makino *et al.*, 1963b).

As a whole, certain karyotypes, in other respects normal, are distinguished by the giant type Y chromosome which may reach the dimensions of a 16–18 or even 13–15 autosome. This variety of giant Y is inheritable; familial observations show that it may be transmitted by phenotypically normal subjects. When we find in their offspring male subjects with a chromosomal aberration, trisomy 21 for example, this does not appear to be modified in any way by the giant Y (Jacobs and Harnden, 1961d). When we find subjects with malformations of unknown origin, the possible determinant role of a giant Y chromosome cannot be advanced without qualification.

With regard to trisomy 21 it is relevant to mention the observations of Makino *et al.* (1963b) of a trisomic 21 girl whose father and paternal grandfather had an abnormally large Y chromosome.

In the absence of inquiries which must be made, the giant Y cannot however now be considered as a factor predisposing to chromosomal aberrations.

II. Varieties with mosaicism

(1) Presumptive $\mathbf{Y}^{\mathrm{ld}}$ *or* $\mathbf{Y}^{\mathrm{ci}}$

A first possibility, that of an X0/X formula and Y deprived of the greater part of its long arm was encountered in a 13-year-old hermaphrodite with dominant feminine habitus. This X0/X$\mathbf{Y}^{\mathrm{ld}}$ observation will be discussed in the chapter on hermaphroditism (Conen *et al.*, 1961c). A similar formula (Ferrier *et al.*, 1962) obtained from blood culture with X0 and XY cells, the Y chromosome appearing in part amputated, was found in a 13·5-year-old girl whose outstanding features were her short stature, obesity with hirsutism, muscular type, wide chest without breast development and abundant hairiness. However, her external genitals were normal though still infantile. Urinary gonadotrophins

were increased but the 17-ketosteroids and excretion of catecholamines were normal. On both sides of a small uterus, with Fallopian tubes, the ovaries were reduced to a typical stroma without signs of germinal activity nor Leydig cells. After discussion the authors considered that the Y deficient by reason of its deletion and the mosaic would have been incapable of Müllerian inhibition and Wolffian stimulation but that it could have directed the phenotype of this subject to the masculine side in certain regions.

Examination of a 61-year-old diabetic female with signs of chromatin-negative Turner's syndrome which could not be confirmed by direct exploration of the internal genitals and who, in addition, showed enlarged clitoris, provided an opportunity for detecting a complex mosaic (Fraccaro *et al.*, 1962c). Three samples of skin from different regions and a blood sample were cultured. Karyotypic study made it possible to distinguish three lines: XXX**Y**^{ld} (skin), X0 (skin) and X**Y**^{ld} (skin and blood). The abnormal Y could be interpreted as a consequence of deletion of the greater part of the long arm or as a short-armed isochromosome of the Y chromosome.

(2) Presumptive giant Y chromosome

Karyotypic study (Van Wijck *et al.*, 1962) of a 46-year-old azoospermic, chromatin-negative male helped to distinguish two varieties of XY cells in line with the normal or oversized dimensions of the Y chromosome.

The authors of these publications rightly take a cautious approach.

We should expect to see rapid and exciting developments along the newly opened road. It is possible that new findings will uncover a series of forms more and more diverging, depending on the type of deficiency of the Y chromosome, from the Turner's X0 syndrome and approaching the normal XY male phenotype.

CHAPTER 12

Hermaphroditism and Pseudo-hermaphroditism

I. HERMAPHRODITISM

Hermaphroditism in the strict sense of the term does not exist in the human species. When during embryogenesis, because of anomalies of development, one sex does not predominate over the other, the individual may be equipped with both masculine and feminine gonads always more or less abnormal, incapable of simultaneously producing male and female gametes and not functional. This abnormal individual is therefore not a hermaphrodite but an intersex individual. While bearing this in mind, we shall use the term "hermaphrodite", which though inappropriate, is hallowed by usage.

Chromosomal study of human hermaphroditism is still far from giving the results which can be expected from it due to the relatively restricted number of cases so far studied. It is also due to the conditions of examination which do not always allow us to extend the karyotypic study to several different regions nor to explore completely the gonads and their adnexa.

Before the cytogenetic possibilities were known the chromatin criterion had been instrumental in separating two types of hermaphroditism: some chromatin-positive, the others chromatin-negative (Barr, 1927). In 25 of these subjects about three-quarters were positive, the others negative (Grumbach and Barr, 1958).

The distribution by anatomical and chromatin types, though chancy because of the small number of cases, gave the following approximations (Guinet, 1960; Putelat, 1957):

1. Unilateral hermaphroditism with ovotestis on one side and ovary on the other: chromatin positive or ovotestis on one side and testicle on the other (two cases of which one was chromatin positive and the other negative);
2. Bilateral hermaphroditism with ovotestis, or ovary and testis, on each side: three-quarters to four-fifths chromatin positive;
3. Alternate hermaphroditism, with ovary on one side, testis on the other: half to three-quarters chromatin positive.

Anatomical records often refer to a rudimentary or immature or Sertolian testicle; an ovary reduced to a stroma of the ovarian type; and sometimes ovotestis on one side with absence of gonad on the other.

The value of these results is reduced by the restricted number of observations, and also by the diversity of the information supplied by the chromatin criterion in presence of mosaicism depending on the region explored. The initial results of cytogenetic study led investigators to distinguish hermaphrodites with and without mosaics.

I. Varieties with mosaicism

(a) X0/XY type

The 3-month-old child studied by Hirschhorn, Decker and Cooper (1960c, d) was an intersex subject with phallus, hypospadias, vagina, uterus, Fallopian tubes and gonads in the position of the ovaries. One of these gonads was studied; it had the features of an immature ovotestis, was made up of a dense stroma resembling ovarian tissue with in the region of the hilus nests of cuboid cells interpreted as structures similar to those of a rete-testis, and near the cortex tubular formations apparently immature seminiferous tubules. Two elements were identified as ovarian follicles. Chromosomal examination (bone marrow) helped to distinguish 60 per cent of the cells with 45 chromosomes and 40 per cent with 46 and established an X0/XY karyotype.

The authors of this observation (Hirschhorn *et al.*, 1960b) then compared it with two others (O.J.Miller *et al.*, 1960b): two subjects of female phenotype with enlarged clitoris, perineal urethra, vagina, uterus and bilateral Fallopian tubes. In these two subjects a seminoma was found on one side, but no gonad on the other. One of these females who had few seminiferous tubules in an ovarian stroma gave the impression of being more a variety of gonadal dysgenesis than an hermaphrodite. The other, more masculinized, whose seminoma contained abundant seminiferous tubules, and also an ovarian stroma, was considered an hermaphrodite.

These observations confirm the idea that gonadal tumours are frequent in intersex individuals.

An observation of Netter *et al.*, (1962) concerns an intersex individual difficult to classify as the authors themselves admit: 14-year subject, with a female perineum but enlarged clitoris, in whom vagina, Fallopian tubes and uterus were normal but with hypertrichosis and masculine body form. Examination of the gonads revealed on the right a Sertolian testicle with Leydig hyperplasia without sclerosis, but with a high value of urinary gonadotrophins (200 mouse units), and on the left a pseudo-gonadal residue suggestive of ovarian dysgenesis in Turner's syndrome. From fascia lata culture an X0/XY mosaic was found.

The above-mentioned paper (Hirschhorn, 1960b) refers to a previous observation (Bloise *et al.*, 1960) presented by its authors as a probable X0 Turner's syndrome (p. 170). It concerns an 8-year-old hypotrophic "boy" whose condition was rendered striking less by the constellation of Turner's syndrome, (low setting of the ears and hairline, high arched palate and naevi), than by signs of intersex: short penis, without urethral meatus, connected to the perineum by a skin web, bifid scrotum with perineal urethra and an orifice leading to hypoplastic uterus, rudimentary testicle in ovarian position on the left and on the right side in symmetrical position, connective tissue rudiments (ovarian stroma). The rudimentary testis was found to contain Sertoli cells, undifferentiated germ cells, spermatogonia and isolated interstitial cells, but these features were associated with feminine signs: separation of the perineal urethra and the vagina, rudimentary uterus with Fallopian tubes and ovarian position of the gonadal rudiments.

The diagnosis of intersex is therefore perfectly tenable. It leads to various hypotheses among others that of an X0/XY mosaic not detected in the bone marrow, perhaps even germinal, or a translocation onto the X, or even an autosome, of the masculine determining factors of Y indispensable for testicular differentiation. If the first hypothesis is verified this subject would be comparable with true XY/X0 hermaphrodites.

A similar observation was reported (Atkins and Engel, 1962b) in a coloured subject of female phenotype. Sexual ambiguity at the age of 13 years was due to phallus of 4 cm, hypospadias, small vagina and prepubertal uterus. In the left labium major there was a testis without spermatogenesis but with Sertoli cells in places and certain vacuolated Leydig cells. Laparotomy revealed a right-side Fallopian tube without gonads. After discussion the authors favour a karyotype 44A + X0 (blood and skin). The buccal cells were chromatin negative. Among the other possibilities that of an undectected X0/XY mosaicism was contemplated.

To these X0/XY examples we must add a further observation (Conen *et al.*, 1961c) of a mosaic of the same type but with deletion of a Y chromosome. After discussion the most plausible interpretation appeared to be a deletion of the major part of the large arm of the Y chromosome and the possibility of translocation of this fragment onto a medium-sized or large chromosome was not ruled out. This intersex was predominantly a female individual but without breast development at the age of 13, with enlarged clitoris, perineal urethral orifice, small vagina, and a rudimentary uterus. On the right an ovary was found with hypoplastic ovarian cortical tissue and stroma of the Turner's syndrome type; on the left a testicle with epididymal tissue. The karyotype was established by examination of blood and bone marrow; cells with 46 chromosomes were in the majority. The nuclei of the buccal cells were chromatin negative.

The authors mention the possible relations between a germinal mosaic and the orientation of female sex differentiation on the X0 side and masculine on the XY (deficient) side.

The same mosaicism was found on examination of an intersex 14-year-old individual with feminine orientated phenotype (Bompiani *et al.*, 1963a) of height 1·31 m, weight 28 kg, with minimal pubic hair growth and absence of breast development. The perineum was ambiguous with a penis of 3 cm, bifid scrotum, perineal orifice leading to the urethra and a vaginal cavity. On the right of a small uterus a small mass of complex histological features in gonadal position was found resembling a gonadal crest, rete-ovary, Leydig elements, tubules, and adrenocortical structure. Intervention for left hernia was said to have revealed a small ovoid formation (gonad?), the subject then being aged 11 months with no further traces of it. The following approximate urinary eliminations were noted in 24 hr: 17-ketosteriods 4·5 mg; 17-hydroxysteroids 3 mg; gonadotrophins, 80 mouse units; normal ACTH test. Examination of blood and skin revealed an X0/XY mosaicism and negative chromatin. The interpretation of this intersex case diagnosed as female pseudo-hermaphroditism is complicated by the results of hormonal and gonosomal study, and made difficult by the absence of information on the left gonad.

The remarks which have been made on X0/XY mosaicism are again relevant here.

(b) XX/XY type

This formula was detected (Waxman *et al.*, 1962a, c; Gartler *et al.*, 1962b; Giblett *et al.*, 1963) in a white intersex individual aged 2 years 4 months with enlarged clitoris, urogenital sinus, uterus and Fallopian tubes with in the position of the right gonad an ovotestis with seminiferous tubules and ovarian follicles, as against an apparently normal left ovary. In addition, this subject had a double coloration of the iris; brown colour on the right of paternal type, hazel colour on the left of maternal type.

A karyotypic study was made. Of 34 cells (blood culture) 33 had 46 chromosomes; among them 7 with XX and 6 with XY formula. The tissue cultures of the two gonads and the skin of the right and left sides of the abdomen gave for the left cell samples mostly XX and for the right mostly XY. The cells coming from the right ovotestis were mostly XY for the testicular portion and mostly XX for the ovarian portion.

Chromatin analysis was run in parallel with chromosomal study (Waxman *et al.*, 1962b) revealing a high correlation between the percentage of sex chromatin masses and the percentage of XX cells, except in the testicular region of the ovotestis and the clitoris, in the view of the authors, because of the small number of cells in the samples of these latter two tissues. These results are presented as additional proof of the hormone-independence of the chromatin body.

These cytogenetic results found an interesting extension in study of the erythrocyte phenotypes revealing the existence of a double population: one reacting with antibodies M, S, C, D, E, e, and the other with the antibodies M, N, s, C, D, c, e.

In addition, the hermaphrodite having inherited in four circumstances only one of the two possible genes since the heterozygotism of his mother had been demonstrated in four loci, ABO, MNSs, Rh and Gm, it is possible that the two fertilized ova had originated from mitosis of an oocyte of the second order, that is, were inherited from the same genome.

This hypothesis of double fertilization prior to elimination of the second polar body is only a possibility; it does not eliminate the hypothesis of fusion of two zygotes, one male the other female.

Moreover, an anomaly of the MNSs system was detected. The mother and maternal grandparents of the hermaphrodite being S− must have been homozygotic for the common allele s. However, the S+ erythrocytes of one of the erythrocytic populations of the hermaphrodite and those of one of his S+ brothers were not agglutinated by the anti-s. The authors consider their observation does not demonstrate any genetic relation between this very rare anomaly in the white race and hermaphroditism.

Double fertilization has also been claimed (Grouchy *et al.*, 1964) to be the origin of an XX/XY hermaphroditism with double population of haptoglobins.

A third example of XX/XY hermaphroditism was also reported.

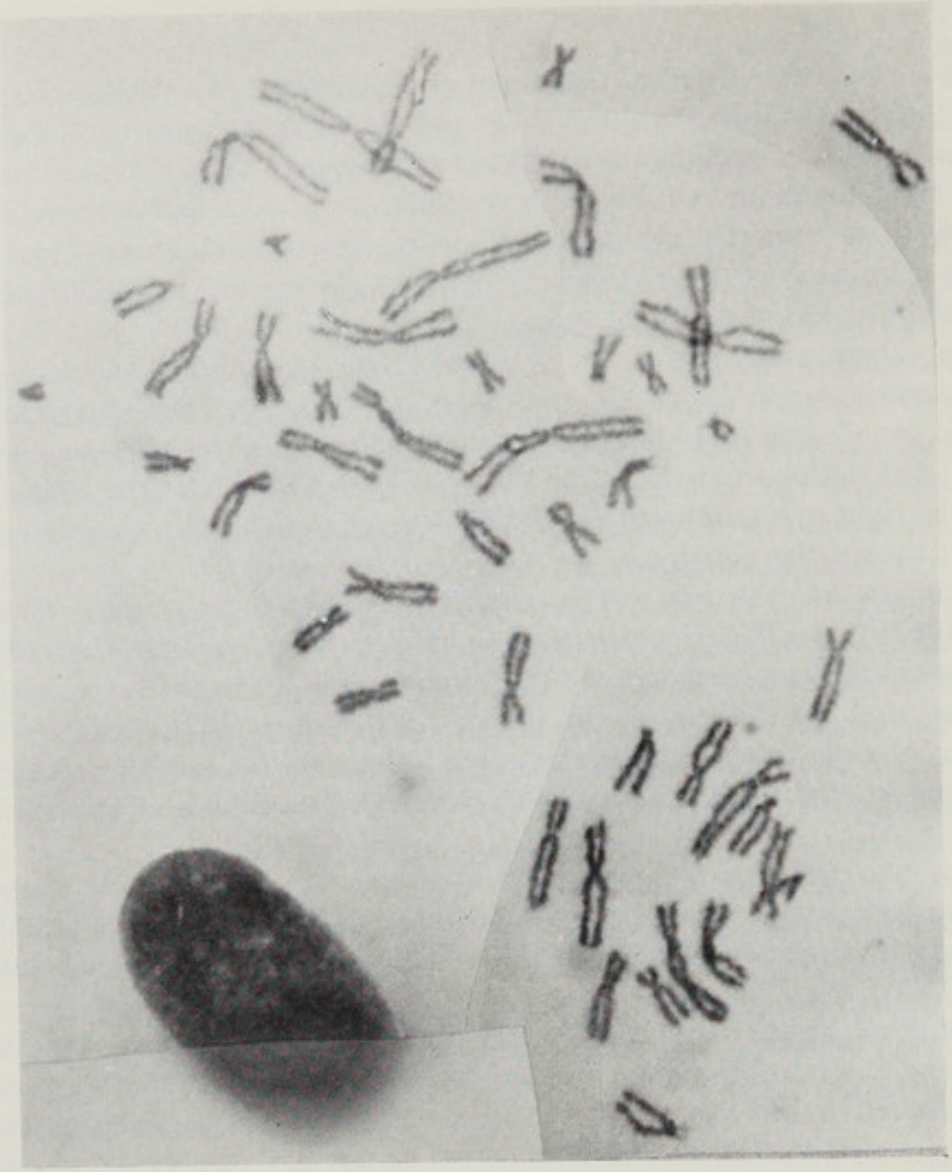


FIG. 12.1. XX/XXY hermaphroditism. Cell with 46 chromosomes.

(c) XX/XXY type

At the age of 4 years the child in whom this type of XX/XXY mosaicism was detected (Turpin *et al.*, 1962c) was distinguished by the ambiguity of his external genitals.

His general morphology was of the masculine type without mental retardation, and his musculature was well developed.

On the other hand, his genital organs were characteristic of so-called "alternate" hermaphroditism: penis of 33 mm on the dorsal side, curved by hypoplasia of its inferior face, glans without meatus, covered on its dorsal aspect alone by the pre-putial cap and traversed on its inferior aspect by a groove leading to an orifice situated at the base of the penis. This orifice of 2·5 mm diameter was the urinary meatus. Urethrography showed a normal urethra which led to the bladder; it did not reveal Müllerian derivatives. This peno-scrotal hypospadias was accompanied by scrotal asymmetry.

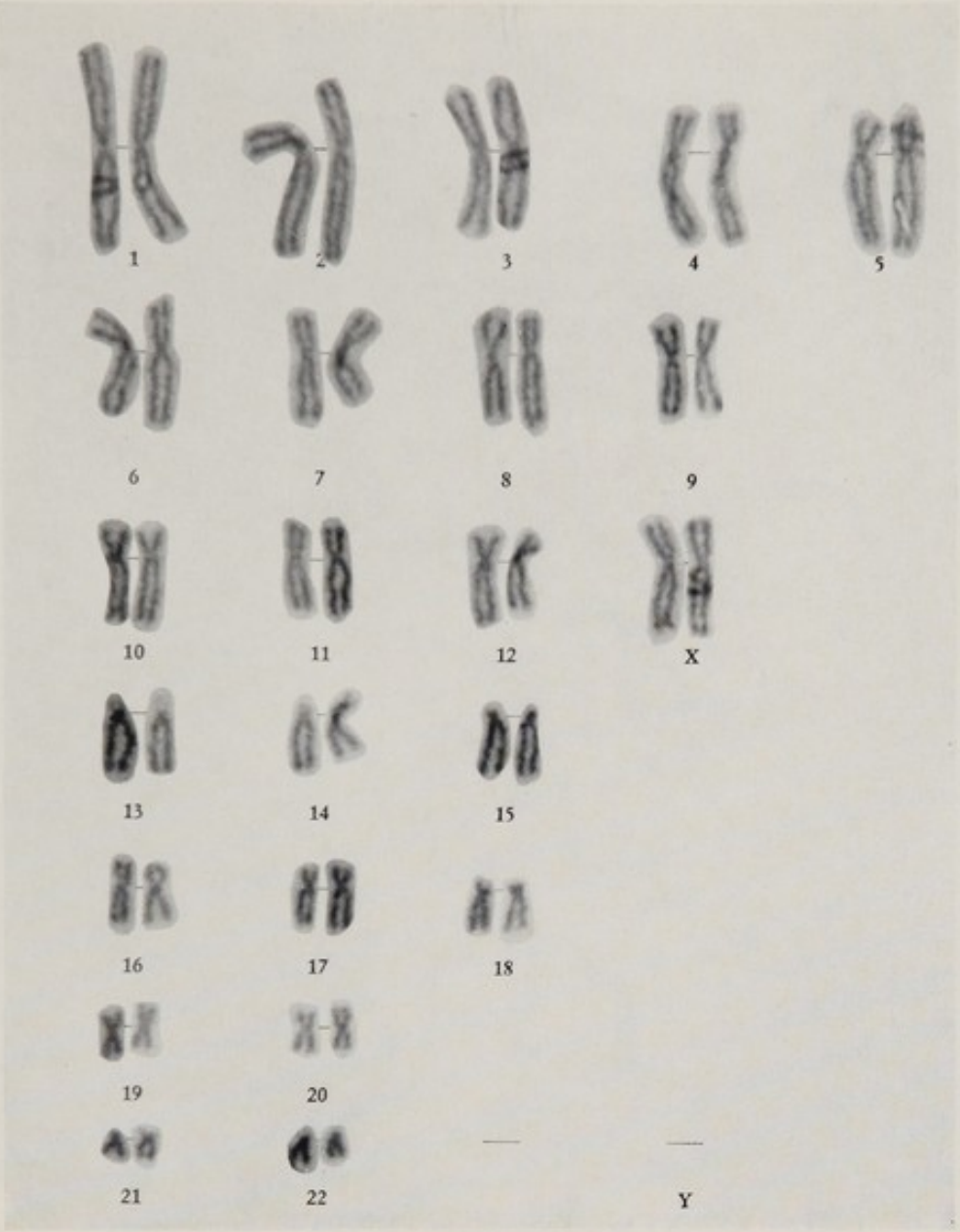


FIG. 12.2. XX/XXY hermaphroditism. Karyotype with 46 chromosomes, XX.

On the left, there was a scrotum with transverse folds in which a gonad the size of a large bean was seen. Histological examination of this gonad enabled the authors to identify immature seminiferous tubules corresponding to the age of the subject with slightly oedematous interstitial connective framework richly vascularized with several arterioles the walls of which were abnormally hyalinized, but despite multiple sections it was not possible to detect Leydig cells.

On the right the scrotum resembled a labium major. It did not contain any gonads and fused into a medium raphe with the left scrotum behind the urinary meatus.

Laparotomy revealed in the right internal inguinal orifice a mass the size of a nut attached to a slightly atretic Fallopian tube. Histological study showed

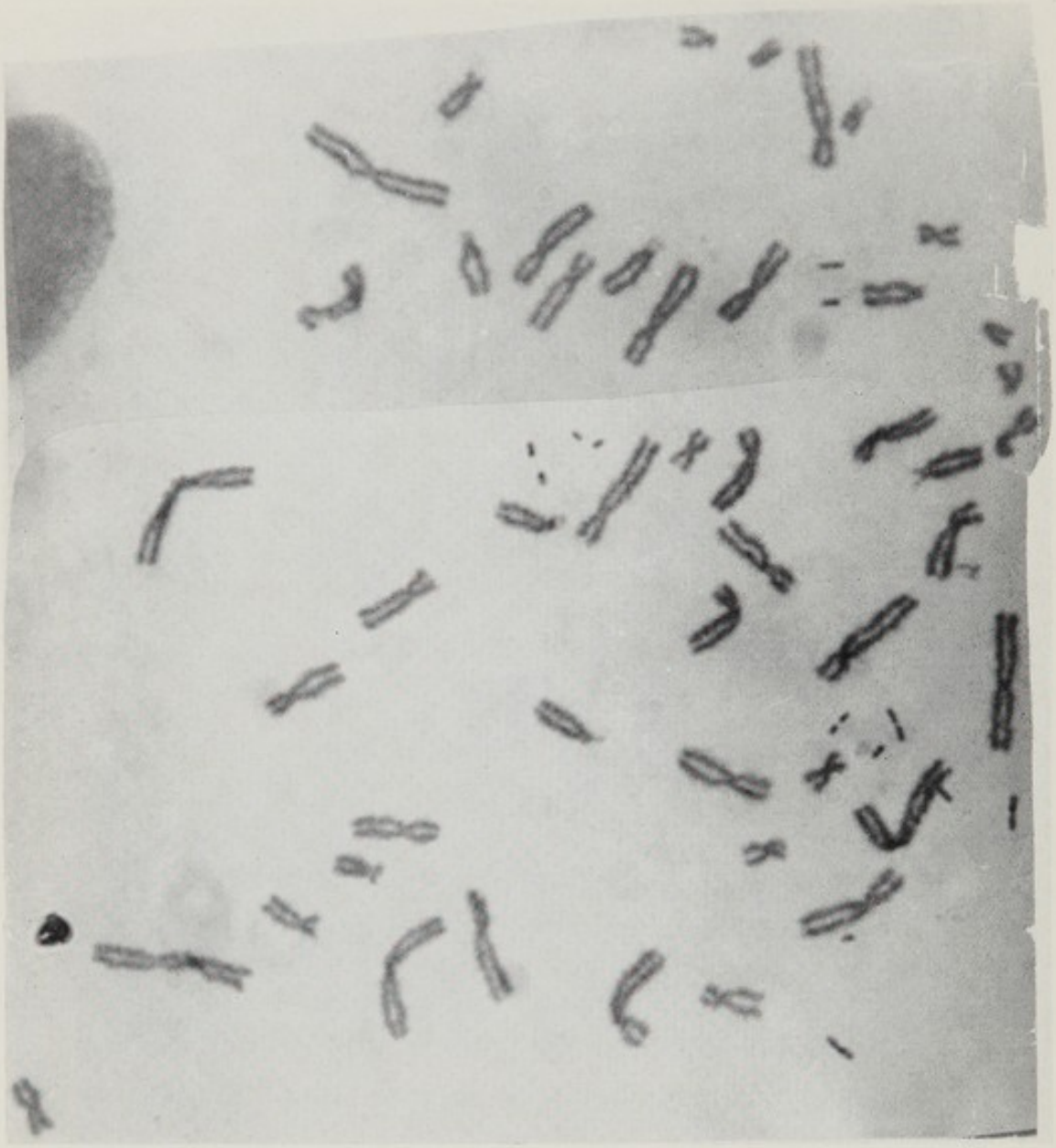


FIG. 12.3. XX/XXY hermaphroditism. Cell with 47 chromosomes.

that the small mass was an ovary with cortical matter containing a considerable number of immature primary follicles enveloped in a normal stroma. There were also growing subcortical follicles containing "follicular liquor" with sometimes even at one pole a germinative eminence populated with granulosa cells. The hilus was normally vascularized containing some typical Berger cells, with Fallopian tube of prepubertal aspect with fringed mucosa. By increasing the number of sections it was possible to find in the meso-salpinx some normal embryonic canalicular remnants and after the Fallopian tube a formation which resembled a rudiment of the uterine horn.

Exploration of the lesser pelvis in particular at the level of the intervesico-rectal space revealed no other anomalies.

This hermaphroditism was therefore characterized on the right by obvious

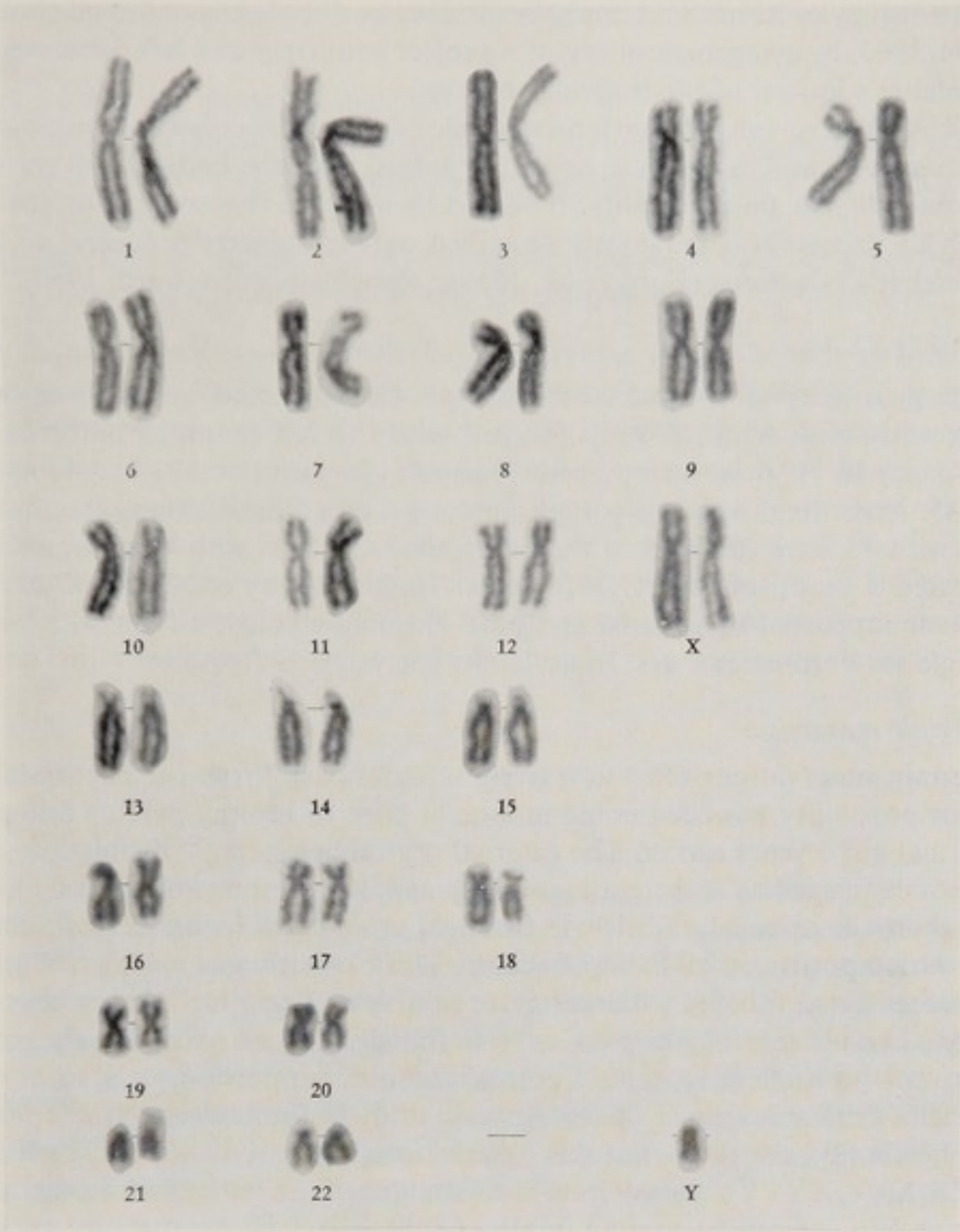


FIG. 12.4. XX/XXY hermaphroditism. Karyotype with 47 chromosomes, XXY.

female adnexa, almost normally constituted and abnormally early follicular growth; on the left by a Leydig testis in which the seminiferous tubules corresponded to the age of the subject. The urinary hormone balance was within normal limits.

This 4-year-old subject was the last of a sibship of four children. The eldest had been operated on for a congenital club foot and bilateral cryptorchidism; the youngest for an angioma of the right arm. No other morbid familial feature was evident.

After checking on presence of buccal chromatin (weakly positive) a karyotypic study was undertaken (fascia lata). It revealed in 98 cells 63 with 46 chromosomes and 35 with 47; the former with an XX gonosomal formula, the latter with XXY.

This variety of XX/XXY hermaphroditism has since been confirmed (Botella-Llusia, 1963) by cytogenetic study of a subject with right and left ovotestes and Klinefelter's lesions of the testicular regions.

XX/XXY hermaphroditism is an example of concordance between gonosomal ambosexuality and in our case, as can be judged from the body of a 4-year-old, anatomo-clinical ambosexuality. It should be noted in this connection that the XX/XXY mosaic was previously described only in subjects presumed to have Klinefelter's syndrome (Ford *et al.*, 1959g; Crooke and Hayward, 1960).

(d) XX/XXX type

The possibility of a mosaic of this type was suggested by an observation (Ferguson-Smith *et al.*, 1960d) grouped with the XX hermaphroditisms (see next page). In 119 bone marrow cells 74 had 46 chromosomes, 22 had 47 and 15 had 45. According to the karyotypic analysis it was difficult to assume that the cells with 45 were X0 but, on the other hand, 11 cells with 47 chromosomes indicated a very probable XXX formula. The possibility of XX/XXX was not however supported by gonadal and jugal chromatin study, all the cells having a single sex-chromatin mass. In no karyotype was a Y found.

(e) Triple mosaicism

Certain observations refer to a triple mosaicism in "true hermaphrodites".

This possibility has been noted in a child born to healthy parents following a normal girl 3 years earlier. The external genital organs were distinguished by perineal hypospadias and right inguinal gonad. Laparotomy followed by histological checks revealed a testicle in the right groin, with ovotestis on the left in the ovarian position with Fallopian tubes. This ovotestis was made up of primitive seminiferous tubules, with spermatogonia, some "possible" Leydig cells and under a capsule several groups of ovarian follicles. Buccal cells were chromatin positive, with single mass and in normal feminine proportion.

After a critical review of the cytogenetic study of four cultures (right gonad, blood, skin [2]), the authors of this observation (Fraccaro *et al.*, 1962g) assume an XX/XXY/XXYYY mosaicism. The formulae of the father and mother were normal. It is interesting to note in view of the buccal chromatin type that the skin cells were practically all XX. The right testicle with 24 cells gave 2 with 45 chromosomes, 12 with 46 XX and 10 with 47 presumptive XXY. The blood gave 129 of 174 cells with 46, 34 with 47 and 7 with 49 (XXYYY) chromosomes.

Another observation difficult to classify (Klevit *et al.*, 1963) concerns a 10-year-old hermaphrodite of short stature, hypospadic with rudimentary uterus and vagina detected by cysto-uretrography with, on the right, a rudimentary Fallopian tube and undifferentiated inguinal gonad with histological features resembling an ovarian stroma.

On the left there were no Müllerian derivatives but a scrotal testis, which when examined revealed a reduced number of interstitial cells and presence of "intratubular bodies" (Bunge and Bradbury, 1961) in the lumens of the dilated tubules consisting of a double bed of "sustentacular" cells, patterns reminiscent of certain degenerating oocytes.

The mosaicism included three cell types (blood and fibroblast culture) interpreted as follows:

(a) the first (45 chromosomes) X0;
(b) the second (46 chromosomes), X and a chromosome suggestive of a 19–20 interpreted as a large-armed isochromosome of Y (or of a Y with partial deletion);
(c) the third (47 chromosomes), in addition to the X and the preceding isochromosome, included a Y with partial deletion of the large arm.

It should be remembered that the intratubular bodies have been seen in at least one case of X0/XY mosaicism and in another with Klinefelter's syndrome.

This observation is difficult to classify. If it is assumed that the right gonadal tissue is an ovarian stroma and that the intratubular bodies of the left testicle are oocytes undergoing degeneration, this observation can be grouped with the X0/XY hermaphrodites. If, on the contrary, it is assumed that gonadal tissue and the left testicle express only abnormal male differentation of the primary gonads, through anomalies of the Y chromosome, this observation merits the definition of male pseudo-hermaphroditism which its authors gave it.

II. Varieties without mosaicism

(a) Type XX

(i) The first karyotypic analysis of a presumptive XX hermaphrodite was reported in 1959 by Hungerford, Donnelly, Nowell and Beck and concerned a coloured intersex subject aged 12 years 4 months predominantly masculine. On the right side in the scrotal position was an ovotestis with Fallopian tube with rudimentary uterine and epididymal tissue; on the left side a testicle with hypoplastic or atrophied seminiferous tubules. Chromosomal examination of the blood of this chromatin-positive subject revealed a large majority of cells with 46 chromosomes of XX formula and a relatively high proportion of cells with 47, the extra chromosome of which could not be classified.

Other similar communications followed this first observation suggesting, with the inevitable qualification that there may be an undetected mosaic, that there exists a variety of hermaphroditism with XX karyotype.

(ii) Seventy-year intersex, predominantly male, subject (Harnden and Armstrong, 1959a; Armstrong, 1955; Armstrong *et al.*, 1957) with right ovotestis with testes predominating but with at least one atretic ovarian follicle and ovary on left. One right and one left arm skin biopsies revealed of 165 cells, 148 with 46 chromosomes. After discussion the authors, excluding an abnormal clone which had appeared in one of the four cultures, postulated an XX karyotype. They confirmed this result on the bone marrow.

The erythrocytes of this subject were devoid of antigen B in spite of the presence of B substance in the saliva. A genetic relation was suggested at that time by the authors (Armstrong *et al.*, 1957) between this anomaly and hermaphroditism.

(iii) Another comparable subject (Ferguson-Smith *et al.*, 1960d), predominantly masculine, had a normal ovary on the left and a testicle on the right recalling that in a Klinefelter's syndrome. Buccal and gonadal chromatin was positive. Of 119 bone marrow cells, 74 had 46 chromosomes and 9 studied were XX. But the possibility also of an XX/XXX formula led the authors to suggest that this case may be a mosaicism (see above).

(iv) This observation was followed by a second example of hermaphroditism predominantly masculine reported by the same authors with ovary on left, ovotestis on the right, and of 86 bone marrow cells, 75 with 46 chromosomes with 16 studied cells giving an XX karyotype with 3 cells containing small similar chromosomal fragments suggestive of a deletion. The authors wonder whether this may not be a residue of a Y chromosome bearing the centromeres and pointing to a Y ~ X translocation.

(v) The interest of these observations is further increased by similar observations.

The hermaphrodite of de Assis *et al.* (1960), predominantly masculine, with urogenital sinus, ovary on left, ovotestis on right, positive jugal chromatin, bone marrow XX.

The hermaphrodite of Gordon *et al.* (1960), at first confused with a chromatin-positive Klinefelter's syndrome, with scrotal testicle on the right with intratubular fibrosis or only Sertoli cells and few Leydig cells; ovary on the left with Fallopian tube, unicornuate uterus; ovary with primary follicles, follicular cyst and one corpus albicans; of 42 bone marrow cells 39 with 46 chromosomes, karyotype XX.

Bilateral hermaphroditism in three sibs (Rosenberg *et al.*, 1962). All three had buccal cells with single sex-chromatin mass, two testicles and two ovaries in scrotal position and Müllerian remnants, XX karyotype, masculine phenotype and social behaviour, with hypospadias reported in the first and third.

The first two of these three brothers examined at puberty showed gynaecomastia. Histological examination revealed in the testicles dysgenesis of the seminiferous tubules (similar to that in Klinefelter's syndrome); in the ovaries, many primary follicles and some atretic ones.

The last brother seen before puberty was distinguished by a hypospadias; testicular histology revealed only apparent reduction in the number of spermatogonia. The normal parents each had a chromatin type and karyotype corresponding to their sex. This familial observation suggests a possible hereditary origin.

A 25-year-old hermaphrodite (Sasaki and Makino, 1960) with female phenotypic orientation but underdeveloped breasts. The perineum was gynoid with peniform clitoris. Only on the right of the bipartite uterus was a gonad found which on examination appeared to be an ovotestis. The results of analysis of the chromatin body are not mentioned. After discussion, the authors support an XX formula (bone marrow).

Nine-year-old hermaphrodite (Crossfield, 1962) of male phenotypic orientation, peno-scrotal hypospadic with left scrotal gonad, half testicle, half ovary; epididymus and Fallopian tube; right scrotal testicle, urogenital sinus, vagina, absence of uterus; chromatin positive and of XX karyotype.

Three hermaphrodites with gynaecomastia but with masculine phenotypic orientation are described in a joint paper (McGovern and Marshall, 1962): one a 15-year-old coloured subject with scrotal ovotestis on the right, left abdominal ovotestis, phallic hypospadias, vagina and rudiment of uterus, XX karyotype; the second a coloured 13-year-old subject with right and left abdominal ovotestes, peno-scrotal hypospadias, urogenital sinus, vagina, uterus and rudimentary Fallopian tubes with XX karyotype; and the third a perineal hypospadic subject with right scrotal gonad, part testicle and part small ovary, on left rudimentary ovary, vagina, uterus and Fallopian tube with XX karyotype.

(b) XY type

An observation of "true hermaphroditism" of XY type (Eliachar *et al.*, 1962) is based on the following anatomical signs at 6 weeks: female perineum with enlarged clitoris, normal female internal genitals apart from the gonads. On the right in ovarian position immature testicle of typical histology; on the left, symmetrical simple nodule the size of a large rice grain with the features of a "very immature" ovary, with large cells situated in a small basket of connective cells resembling oogonia. In the view of the authors a fuller inquiry into possible mosaicism by chromatin and chromosomal analysis is necessary.

Discussion

The distribution of karyotype in the observations so far published does not indicate any agreement between the gonosomal formula and the anatomo-clinical type of intersex.

Cytogenetic studies, although they are only in their initial stages, suggest some interesting reflections on the possible origins of human hermaphroditism.

(a) The X0/XY subjects are far from being a single type. The orientation of the phenotype, when the age of the subjects enables it to be defined, is sometimes masculine but more often feminine. Most of the observations refer to a large clitoris or a short phallus and a perineal urethral orifice, vagina with uterus and Fallopian tubes often hypoplastic. Sometimes the features of the gonads are uncertain with perhaps an ovotestis or testicle without spermatogenesis on one side, with no contralateral gonad. Sometimes there exist two gonads where the ovaries should be and examination reveals an ovotestis in only one of them. Finally, sometimes there exists one ovary on one side and a testis on the other.

In the latter case the anatomical descriptions are not concordant: testicle on the right, Sertolian with Leydig cell hypoplasia and left pseudo-gonadal residues evocative of Turner's syndrome; testicle on left, Sertolian with undifferentiated germ cells, some interstitial cells and rudiments of ovarian stroma on right; ovary on right with hypoplastic cortical matter and stroma of Turner's syndrome; or testicle on left with epididymal tissue.

It is interesting to relate these differences to other interpretations to which the X0/XY types have given rise apart from hermaphroditism, cases considered as variants of Turner's syndrome (p. 210) or of testicular feminization (p. 210),

observations of males with testicle on one or both sides (p. 231) and testicles without spermatogenesis. This diversity is amenable to several explanations.

The first may refer to uncertainties which for technical reasons are involved in any diagnosis of mosaicism.

Certain X0/XY cases may correspond in fact to more complex mosaics because of one or more different supernumary clones or unrecognized translocations. Excluding this possibility it would be necessary to assume that the phenotypic varieties reflect varieties of regional distribution of X0 and XY clones. This distribution at the level of the primary gonads is certainly very important. It determines their future and hence the development of the adnexa which depend on them.

This explanation appears to us to be highly valid. It does not exclude, moreover, the individual role of the genotype. This permits possibilities of variations since the X0/XY aberration must fit the genotype environment peculiar to each individual.

(b) The XX observations form a less heterogeneous whole. If we exclude the observation (Sasaki and Makino, 1960) of a suject of female phenotypic orientation in whom only a single gonad was found (right ovotestis), all the XX cases concern intersex individuals of male phenotypic orientation with two gonads. In most cases the right gonad is an ovotestis, the left an ovary. It is sometimes noted that this is a normal ovary.

Other descriptions refer to several anatomical variants.

Instead of an ovotestis there is a testicle with features recalling those in Klinefelter's syndrome. The authors of one of these observations (Ferguson-Smith *et al.*, 1960d) do not dismiss the possibility of an XX/XXX mosaicism. Other authors (Gordon *et al.*, 1960) from the results of study of the bone marrow opt for an XX karyotype. Instead of the left ovary another subject (Hungerford *et al.*, 1959) had a testicle with hypoplastic or atrophied seminiferous tubules. The XX diagnosis did not exclude a mosaicism, examination of the blood revealing a high proportion of cells with 47 chromosomes.

Finally, a familial observation affords an example of bilateral hermaphroditism, three sibs each with two ovaries and two testicles (Rosenberg *et al.*, 1962).

Because of these modes, XX intersex individuals are also open to different interpretations.

Some are considered as possible mosaics in an XX guise; or as a consequence of a Y ~ X translocation revealed by the presence in certain cells of a small chromosome fragment with a centromere suggestive of a Y residue (Ferguson-Smith *et al.*, 1960d).

Others, such as those of the familial observation above without an apparent anomaly of the XX karyotype (Rosenberg *et al.*, 1962), are explicable in terms of an indiscernible structural anomaly or a genic anomaly.

There are no doubt other XX hermaphrodites without apparent karyotypic anomalies, human replicas of hereditary animal hermaphroditism.

(c) Two other karyotypic varieties are particularly worth noting. Intersex of XX/XXY masculine type offers an example of concordance between gonosomal

and anatomo-clinical ambosexuality. This observation also shows that in absence of anatomical proof we cannot be content with the diagnosis of Klinefelter's syndrome in presence of such mosaicism.

Intersex XX/XY derives its origin from double fertilization or fusion of two zygotes, one male, the other female. Gonadal tissue cultures reveal concordance between the anatomical and gonosomal type: XX formula of the cells originating from the ovary, with chiefly XY formulae of the cells of the testicular region and mostly XX in the ovarian region of the ovotestis.

In conclusion, the observations reported may be divided into three categories: prozygotic hermaphroditism of genic origin; zygotic hermaphroditism, through double fertilization or fusion of two zygotes with a double red cell population; or metazygotic hermaphroditism with mosaicism through abnormal mitotic segregation.

II. PSEUDO-HERMAPHRODITISMS

I. Male pseudo-hermaphroditism

(a) XY type

Many intermediate cases come between the two extremes one external, the other internal.

The first is marked by external predominance. The Müllerian apparatus has been correctly inhibited, the genital organs are more or less well developed but in the Wolffian sense. Testicles are histologically normal before puberty, then deprived, apart from a few exceptions, of elements of the seminal line. The perineum is feminine or feminoid. This variety may be explained by the action of the anti-Müllerian principle and deficiency of the principle of Wolffian stimulation. Puberty is feminine (testicular feminization).

The second is marked by internal predominance. These "males with uterus" have normal external genital organs. It is as though the inadequacy of the anti-Müllerian principle has allowed an utero-tubular process to develop and as though the principle of Wolffian stimulation had then acted on the development of the urogenital sinus. These subjects will display masculine puberty.

Intermediate cases with incompletely masculinized or ambiguous perineum come between these two varieties. The direction puberty will take is then unpredictable.

(i) The majority of male pseudo-hermaprodites who have formed the subject of cytogenetic studies have been XY (Jacobs *et al.*, 1959d; Lejeune *et al.*, 1960d; Puck *et al.*, 1960; Lennox, 1961c; Chu *et al.*, 1961b; Alexander and Ferguson-Smith, 1961; Bompiani *et al.*, 1961; Makino *et al.*, 1962e; Giraud *et al.*, 1962) and chromatin negative. Other studies have not verified the previous hypothesis of the XXY formula (Danon and Sachs, 1957; Taillard and Prader, 1957; Peterson and Bonnier, 1937).

They, however, confirm a view supported since 1942 by Severinghaus from analysis of the spermatocytes of one of his patients, even before the normal human karyotype was known.

(ii) Testicular feminization

Male pseudo-hermaphroditism, "testicular feminization" in particular, has been the subject of familial studies. In these families when classifying the subjects according to their chromosome sex, that is, the intersex individuals among boys, we find that the sex ratio is very close to unity, that is, normal.

Two possibilities have been considered (Bonnier, 1938; Grumbach and Barr, 1958; Lenz, 1959a). One is that of a recessive disorder associated with an X chromosome, the other a sex-controlled autosomal dominant disorder.

Since the subjects are sterile it is not easy to decide between these two hypotheses. Study of the localization of the responsible gene on the chromosome has therefore been based on X chromosome-linked marker genes (Lenz, 1957) since a demonstration of a connection with daltonism, for example, may solve the problem.

Two observations have recently been considered from this point of view (J.S.S. Stewart, 1959d).

One concerns two boys, one normal and not colour-blind, the other an XY pseudo-hermaphrodite with extreme deuteranomaly. The deceased mother must therefore be presumed to have been heterozygous for the recessive character, colour blindness, in the hypothesis of an X chromosome-linked recessive pseudo-hermaphroditism.

The other concerns four children: normal girl; XY pseudo-hermaphrodite with normal colour perception; normal boy but with deuteranomaly; and probable male pseudo-hermaphrodite, chromatin negative and with deuteranomaly.

This distribution suggests that, even if the loci for pseudo-hermaphroditism and colour blindness are both on the X chromosome, their linkage cannot be very close since a recombination gives the appearance of a random segregation.

These findings encouraged study of similar observations.

Other observations of testicular feminization plus sex-linked hereditary disorders have been published: classical haemophilic A (Nilsson *et al.*, 1959) and anodonty (Pfeiffer, 1962a).

Another observation (Sukumaran and Shah, 1962) concerns a familial study of an autosomal marker gene, an abnormal haemoglobin. A male pseudo-hermaphrodite had haemoglobin of normal adult type and a slow moving fraction. This type identified as haemoglobin A + D was found in the mother and a brother in other respects normal, whereas the father and another brother had normal adult haemoglobin. This coincidence in the character of D haemoglobin in the pseudo-haemaphrodite and his normal brother is enlisted by the authors in support of the thesis of autosomal transmission of male pseudo-hermaphroditism.

Lipoid hyperplasia of the suprarenals as in Prader's case, whether the gonads are ovaries or testicles, is characterized by external genital organs of feminine type. However, this differentiation among XY subjects is not distinctly feminine and gives a type of intersexuality (Prader and Siebenmann, 1957). This disorder is no doubt autosomal recessive and related to a deficit of C-20, 22-desmolase (?).

Another familial observation (McKusick, 1962) covering three pseudo-hermaphrodite brothers, all XY, also provides arguments supporting existence of an autosomal recessive variety of male pseudo-hermaphroditism.

The marker gene used was the Xg blood group. The mother was Xg (a+), a normal son Xg (a−), a pseudo-hermaphrodite Xg (a−) and the other two children—pseudo-hermaphrodites—were Xg (a+). The author of this publication considers that this observation and certain others which used colour blindness or haemophilia A as markers suggest that the gene for male pseudo-hermaphroditism segregates independently of these marker genes and that it is autosomal.

(b) Deviant types

We may tentatively classify some atypical observations for which the authors emphasize their exceptional nature.

(i) On the one hand, the observation of Shah *et al.* (1961) of male pseudo-hermaphroditism with XX karyotype and intra-abdominal testicles. Only the skin was studied so that a possibility of mosaicism cannot be dismissed. Two other XX male subjects (Court Brown *et al.*, 1964) raise a similar problem.

(ii) On the other hand, observations of mosaics.

1. Three observations of the X0/XY type have been considered by authors as possible variants of testicular feminization.

The 22-year-old subject of feminine phenotype described by Willemse *et al.* (1962) was primarily amenorrhoeic with enlarged clitoris, the uterus and breasts were rudimentary and two gonads with seminiferous tubules without spermatogenesis were found in ovarian position, with Leydig cells on the left and rare tubules and clusters of Leydig cells in a connective tissue with nerve elements on the right.

The patient of Ferrier *et al.* (1962) had a masculine phenotype with hypospadias, rudimentary uterus, Fallopian tubes and gonads both containing tubules, some with a few spermatogonia and a few interstitial cells.

These two observations are distinguished by the size of the Müllerian rudiments.

The third example reported by Grouchy *et al.* (1963h) concerned a 4-year-old child with vulviform hypospadias, gonads in lateral scrotal folds, urogenital sinus and "horseshoe" kidney. The parenchyma of the right testicle was immature and more or less normal, the left was not biopsied but from its morphology the authors consider it unlikely that it contained ovarian tissues. Peripheral blood culture showed an X0/XY mosaic while examination of the fascia lata showed only X0 cells.

The observation of a 9-year-old child of feminine phenotype but whose perineum was ambiguous is pertinent here (Bottura and Ferrari, 1962a). Examination of the genital apparatus revealed a 4–5 cm phallus without urethral meatus, a rudimentary vagina and uterus, permeable Fallopian tubes at X-ray, the right one being longer than the left, with no gonad on the left and an immature testicle on the right. No chromatin bodies were found in the many regions explored (oral smears, abdominal and phallus skin, smooth muscle, cells within semini-

ferous tubules). Of 51 fresh bone marrow cells, 45 contained 45 chromosomes and appeared to be X0. However, the possibility of an X0/XY mosaicism was not excluded.

In an infant whose salient features were rudimentary phallus with urethral meatus at the base, bifid scrotum with left testicle and negative buccal chromatin, an X0/XY mosaicism was found (blood) (Conen and Erkman, 1963b).

Intervention for strangled right inguinal hernia was followed by excision of a vagina with uterus, and right Fallopian tube and gonad. The latter contained neither primary follicles nor identifiable tubules.

2. Another case (Schuster and Motulsky, 1962) was of the X0/XX/XY type. The case in question was an intersex individual whose external organs were ambosexual: penis and normal labia majora, vaginal urethral orifice, atrophy of the labia minora and greatly reduced vaginal orifice plus absence of mammary development. Furthermore, there was a testis in right ovarian position, without elements of the seminal line and with ducti deferentes on each side. The impossibility of identifying the fibrous structure in the left ovarian position and the presence of Fallopian tubes on each side preclude an unambiguous definition of this intersex variety.

3. An X0/X**X**^{ld}Y variety described by Miles *et al.* (1962c) concerned a 13-month child. The perineum was ambiguous with a phallus of 1·5 cm, urethral meatus and vaginal introitus. Laparotomy at 6 months followed by biopsy showed a uterus with Fallopian tubes and in ovarian position a gonad which on each side was a testicle with infantile seminiferous tubules and rete-testis. The cells studied from buccal, skin and gonad samples were sex-chromatin negative. Karyotypic study, after discussion, led the authors to conclude that the probable formula was X0/X**X**^{ld}Y. In fact, the small extra chromosome with a submedian centromere was suggestive of an X chromosome reduced by deletion of the long arm.

4. An example furnished by Warkany *et al.* (1962a) of X0/XXXY mosaicism has been verified on a number of occasions in a masculine negro infant by skin and blood cultures. The perineum was masculine but the short penis had a ventral "palmature" and capped prepuce, with normal urethra and meatus. Surgical exploration at 2 weeks revealed, uterus, bilateral Fallopian tubes, bilateral inguinal hernia, "infantile" testicle on left, absence of gonad on right, calyx and right urethral dilatation with ouracal cyst. Physical and mental development at 16 months was considered satisfactory. Pending the results of further search for a right gonad this observation was placed in the group of male pseudo-hermaphrodites.

5. Observation of triple mosaicism with Y anomalies (Klevit *et al.*, 1963) was discussed in the section on hermaphroditism (pp. 238 and 239).

6. In an infant with phallus, urogenital sinus, bifid scrotum and feminine internal genitals, two testicles were found in ovarian position. The buccal and urogenital chromatin-negative cells contrasted with the presence of drumsticks. Skin, testicular and muscle culture revealed an X0 formula; blood culture an X0/XX mosaicism (Conen and Erkman, 1963b). This observation diverges from the X0/XX type normally suggestive of Turner's X0 syndrome (p. 180).

II. Feminine pseudo-hermaphroditism

This intersex variety whose name is justified by its XX karyotype includes several aetiological varieties.

(a) Congenital hyperplasia of the suprarenals, while producing in the boy early pseudo-puberty, gives in the girl an XX pseudo-hermaphroditism.

While the internal genitals are feminine and normal, the external genitals of these girls are more or less masculinized with enlarged clitoris and developmental anomalies of the urogenital sinus. The familial character of this intersex variety is well established and suggests with good reason its recessive and autosomal nature.

However, the existence of several bioclinical types leads us to distinguish several genic types.

The most common—the pure virilizing—form increases urinary elimination of 17-ketosteroids and pregnanetriol (precursor of hydrocortisone) at the expense of the 17-hydroxysteroids which are reduced. These anomalies result from congenital deficit of a suprarenal enzyme, 21-hydroxylase, necessary for biosynthesis of hydrocortisone (Childs *et al.*, 1956; Wilkins, 1961).

The hypertensive form reflects insufficiency of 11-hydroxylase entailing insufficiency of hydrocortisone and accumulation of desoxycorticosterone and 17-hydroxy-11-desoxycorticosterone.

The form with the salt depletion syndrome is thought to result from production of a hormone promoting loss of salt (Debré-Fibiger syndrome). It is probable that genic varieties with familial autonomy of bioclinical types correspond to the enzymatic varieties.

(b) Feminine pseudo-hermaphroditism due to congenital hyperplasia of the suprarenals must be distinguished from pseudo-hermaphroditism due to hyperandrogeny of maternal origin (virilizing tumour of the ovaries; treatment during pregnancy with substances with androgenic powers). Here, there is no excess of 17-ketosteroids and pregnanetriol in the urine. In addition, the condition ceases to develop after birth.

(c) Other rare examples of feminine pseudo-hermaphroditism cannot be related to the preceding forms. While the karyotype enables us to distinguish them from male pseudo-hermaphroditism, histological examination of the gonads permits us only to exclude hermaphroditism. The problem they raise is in the last analysis one of morbid anatomy.

CHAPTER 13

Monozygotic Twinning and Chromosome Aberrations (Heterokaryotic Monozygotism)†

AMONG the possible consequences of chromosome aberrations a new type of pathological monozygotic twinning today merits consideration. This new type was discovered by study of a pair of twins—a normal XY boy and an abnormal X0 girl (Turpin *et al.*, 1961e, 1963b, 1965a). This original description has since been extended by that of a case of diplo 21-triplo 21 twinning, and from other authors by an XY–X0 pair and of two XX–X0 pairs. We have given the name "heterokaryotic monozygotism" to this new type of twinning.

XY–X0 variety

I. (Obs. 1). The first couple who initiated this study consisted of a boy and a girl aged 17 years, the issue of a pregnancy defined as monochorionic from the macroscopic aspect of the placenta. The boy was normal: 56 kg, 1·65 m in height and with an IQ of 125. The girl was abnormal: 40 kg, height 1·42 m and with difficulties in learning, with an IQ of 106. She attracted attention by various dystrophies: poorly contoured ear lobes, low-set with adhering lobule; webbing of the neck with typical wings; upper limbs deformed by cubitus valgus. But the most striking feature was that despite her age this patient had never menstruated. This primary amenorrhoea was associated with absence of secondary sex characters; the breasts and pubic and axillary hair were not developed. The external genitals were normal feminine but an exploratory laparotomy revealed total absence of right and left gonads, although utero-tubular development was normal.

This gonado-somatic dysgenesis was accompanied by abnormally high urinary elimination of gonadotrophins of more than 100 mouse units in 24 hr and by the absence of nuclear chromatin bodies in the cells at interphase in jugal cells and in fascia lata culture.

The results of these examinations led us to conclude that this girl was suffering from Turner's syndrome and therefore to go on to a karyotypic investigation of both twins and also of the type of twinning. It should be noted that two cousins four times removed of the mother of these children had themselves also

† This particular chapter has been slightly modified and brought up to date before its translation into English.

TABLE 13.1

Father	Mother	Sister A	Sister F	44A + XY♂ ♀ 44A + X0	Probability of phenotypic concordance
Erythrocyte phenotypes					
A 1	0	0	0	A 1	1/2
MNSS	MNSs	MNSS	MNSS	MNSs	1/4
P 1	P 1	P 1	P 1	P 1	0·85 (*)
Lu (*a*−)	Lu (*a*−)	Lu (*a*−)	Lu (*a*−)	Lu (*a*−)	1
CcDee	ccddee	ccddee	CcDee	CcDee	1/2
kk (**)	kk	kk	kk	kk	1
Fy (*a* + *b* +)	Fy (*a* + *b* +)	Fy (*a* + *b* +)	Fy (*a* + *b* +)	Fy (*a* + *b* +)	3/8
Jk (*a* + *b* +)	Jk (*a* + *b* −)	Jk (*a* + *b* +)	Jk (*a* + *b* +)	Jk (*a* + *b* −)	1/2
Au (*a* +)	Au (*a* +)	Au (*a* +)	Au (*a* +)	Au (*a* +)	0·75
Xg (*a* +)	Xg (*a* +)	Xg (*a* +)	Xg (*a* +)	Xg (*a* +)	0·76
Serum phenotypes					
Gm (*a* + *b* + *x* + *e* + *r* +)	Gm (*a* − *b* + *x* − *e* + *r* −)	Gm (*a* − *b* + *x* − *e* + *r* −)	Gm (*a* − *b* + *x* − *e* + *r* −)	Gm (*a* + *b* + *x* + *e* + *r* +)	1/2
Gm-like (−)	Gm-like (−)	Gm-like (−)	Gm-like (−)	Gm-like (−)	1
Hp 2–2	Hp 2–2	Hp 2–2	Hp 2–2	Hp 2–2	1
Inv (1 − *a* − *b* +)	Inv (1 + *a* + *b* +)	Inv (1 + *a* + *b* +)	Inv (1 + *a* + *b* +)	Inv (1 + *a* + *b* +)	1/2
Salivary phenotypes					
AH	H	H	−	AH	Se: 5/8
$Le^a.Le^b$	$Le^a.Le^b$	$Le^a.Le^b$	Le^a	$Le^a.Le^b$	Le: 0·87
(Se.Le)	(Se.Le)	(Se.Le)	(sese.Le)	(Se.Le)	II = 0·00074

(*) Probability calculated from the genic frequencies; $P = 0{\cdot}54$ and $p = 0{\cdot}46$. In addition, the two twins were found to be negative to an antibody probably recognizing an antigen of the *P* system corresponding perhaps to a recessive gene. All the other members of the family were positive for this antigen (about 1% of white subjects are negative. Sanger and Race). This observation greatly strengthens the notion of identity but cannot be used statistically.

(**) kk = K − k +, Kp (*a*−), Kp (*b*+).

borne twins, one a boy and a girl, the other of twins of unknown sexes. Familial coincidence of twinning and certain chromosome aberrations does not appear to be always fortuitous.

(a) Cytogenetic study of the fascia lata cells and then of the skin cultures confirmed the anatomo-clinical dissimilarity. This study confirmed the presumptive karyotypes of the boy as 44A + XY and of the girl as 44A + X0.

These cytogenetic analyses disclosed no sign of mosaicism.

(b) An investigation of the criteria of monozygotism was made in parallel.

(i) The criteria which are influenced by the sexual phenotype of the individual or by constitutional anomalies were here of little interest. However, dermatoglyphic analysis although it does not escape sex control revealed concordances between the finger tip patterns of both hands, finger for finger for the second, third, fourth and fifth and for the first of the left hand; between the patterns of the phalanges and middle phalanges, finger for finger, of both hands and between the arrangement of the three flexion creases and the axial triradius in position t, absence of a pattern in space 11, presence of ridges emanating from the triradii a and b on both hands and orientation of the ridges of the thenar eminence. The following discordances were noted: double loop on the right thumb in the XY subject and single ulnar loop in the X0, a pattern in space 7 on both hands in the X0, a pattern in space 9 of both hands in the XY subject and hypothenar radial loop on the left hand of the X0 girl. Discordances of patterns in space 11 imply differences in orientation of the ridges emanating from the triradii c and d and hence the difference in the total index: 20–21 in the XY and 26–26 in the X0 subject.

The distinctive dermatoglyphic features of X0 subjects are still insufficiently known for it to be possible to relate these discordances to this dysgenesis. This possibility, however, cannot be dismissed. It was invoked to explain a difference between the number of creases in the finger dermatoglyphs of an XY boy and his twin X0 sister (Obs. 2).

(ii) Furthermore, two criteria retained their value: on the one hand, familial analysis of the erythrocyte, serum and salivary phenotypes and, on the other, reciprocal skin grafts.

(1) Erythrocyte, serum and salivary analysis established the identity of the two twins for all the factors studied and allow us to consider that the probability of such concordance assuming dizygotism would be of the order of $p = 0{\cdot}00074$.

	X0	XY	Total
1st series	12	2	14
2nd series	15	6	21
3rd series	12	13	25
4th series	5	6	11
	44	27	71

$\chi^2 = 7{\cdot}48$ for $r = 3$, $P \neq 0{\cdot}05$.

(2) The criterion of reciprocal skin grafts showed that they behaved as autoplasties and that the cells of the graft persisted 77 days after the test in the X0 (graft of XY karyotype) and 358 days after the test in the XY (graft of X0 karyotype) and that these grafts did not produce immunological reactions. This supplementary verification was made with the aid of three methods: anti-erythrocyte (routine technique), anti-leucocyte (technique of André, Dreyfus and Salmon), and anti-platelet antibodies (technique of Salmon and Schwartz).

(iii) A little time later a study of the blood karyotype of these two twins became possible revealing in the X0 girl the apparently exclusive presence of XY cells and in the XY boy the presence of two X0 cells for 18 XY.

This result in view of the common circulation which is set up between monozygotic twins from the start of the fourth week (see p. 258) which follows fertilization was not surprising. Although the proportion in the XY boy's blood of about 10 per cent of X0 cells would have no doubt been sufficient to have given a double erythrocyte population it was decided to make a new examination to eliminate the faint possibility of chimerism of dizygotic twins.

This examination consisted of two investigations.

(1) The first, measurement of the intensity of agglutination, was made with five antisera: A, M, N, D and B, and red cell suspensions in low concentrations. Artificial control preparations with 10 per cent non-agglutinable red cells were also used. This technique did not reveal an obvious double population either in the boy or in the girl with Turner's syndrome.

(2) However, since agglutination of the red cells by anti-M serum was not complete, a second check was run by the qualitative method of Filitti-Wurmser. It showed that the agglutinability of the red cells in the Turner's syndrome was 90 per cent but in view of the results obtained in the controls this finding had no statistical value. This double check confirmed the absence of a double population, that is, of a chimera in both twins.

It confirmed the hypothesis of dissimilar monozygotic twinning with XY karyotype in one and X0 in the other (skin and fascia lata). Blood culture revealed an exchange of cells consequent on joint circulation peculiar to monozygotic twins and with no apparent phenotypic consequences.

II. (Obs. 2). Since this report first published in 1961, a second pair of the same type as that we have just described has been observed (Dent and Edwards, 1963b; Edwards *et al.*, 1966).

A 15-year-old girl with amenorrhoea attracted attention by her height of 1·40 m for a girth of 1·46 m, her weight of 29 kg and absence of secondary sex characters, breasts and sex hair. This retardation was in part corrected by treatment based on oestrogens and progesterone: menstrual periods appeared and the breasts developed and in 5 years her weight rose by 12·7 kg and her height by 11 cm. The diagnosis of Turner's syndrome was made but at 21 years it was reflected only in the short stature without webbed neck, and widespread freckles, mainly on the arms.

The twin brother of this subject with Turner's syndrome, muscular with deep voice and bushy beard, had clinically normal testicles and cords. Married for a year he had no children, even though he had not used contraceptives. He de-

viated from normal by his height of 1·56 m and by a "curious ability to draw his shoulders behind his back" and a discordance in colour of the irises.

The following criteria were involved.

(a) Masculine skeletal characters for the boy and feminine for the sister, despite some similarities.

(b) Identical erythrocyte and serum groups.

(c) Dermatoglyphs very similar except for left index finger with radial loop in the boy and large ulnar loop in the girl, and in the numerical value of the digital patterns, index of 115 for the boy and 132 for the girl.

This last character bore the stamp of Turner's syndrome for the authors who considered that this index—normally lower in girls than in boys—was above that in girls with Turner's syndrome.

(d) ECG normal and identical in both twins.

(e) In the girl negative buccal chromatin with no drumsticks in the blood.

(f) Discordant iris pigmentation: in the girl both blue, in the boy blue on the right side like those of his sister with an almost completely brown–green iris on the left side. To explain this discordance the authors, in relation to a presumptive X0/XY mosaicism, tentatively advance the hypothesis of Y chromosome-linked genes of iris colour.

(g) Karyotypes.

Subject with Turner's syndrome:

blood, lymphocytes	45 X0 7 XY
	36 X0 2 XY
fibroblasts (skin)	59 X0 1 XY?

Twin brother:

blood (lymphocytes)	10 X0 1 XY?
	27 X0 –0–
fibroblasts (skin)	62 X0 –0–
	81 X0 –0–

XX–X0 Variety

A second variety of this pathological twinning is that of monozygotes in which one female is normal and the other partner shows Turner's syndrome.

I. (Obs. 3). The first observation of this type was reported in 1963 (Mikkelsen *et al.*). The pair issued from a diamniotic, monochorionic pregnancy, one a normal girl, the other a girl distinguished by signs of Turner's syndrome.

The first, normal girl, had positive buccal chromatin (51 of 200 cells with single chromatin mass).

The second, with features of Turner's syndrome, showed negative buccal chromatin (200 cells). In these two children karyotypic analysis disclosed a blood and skin X0/XX mosaicism. The proportions of the two cell types with 46 and

45 chromosomes were as follows. For the skin (normal twin) 25 XX, 21 X0 and 10 XX, 9 X0 (culture at 16 weeks), and for Turner's syndrome 4 XX, 24 X0. For the blood the normal twin showed 31 XX, 17 X0 and the Turner's syndrome subject 33 XX, 15 X0.

The child suggestive of Turner's syndrome was both mentally and physically retarded with convulsions and webbing of the neck. She died at 3 years 10 months. Post-mortem examination showed that the external genitals were normal as were also the uterus and tubes. Examination of the gonads in ovarian position, showed in an ovarian stroma the presence of primordial follicles. The following features were also noted: cerebral lesions of pachygyria and microgyria which are frequent malformations in Turner's syndrome. The heart showed an inter-auricular communication and the urinary tract a horseshoe kidney.

II. (Obs. 4). A second similar case has since been reported (Edwards, 1965; Edwards *et al.*, 1966) concerning a pair of female twins born in the 36th week of pregnancy. Their German mother of 25 years and Pakistani father of 43 years already had a normal girl. Study of the erythrocyte and serum groups and skin pigmentation of the twins ruled out illegitimacy.

At first sight the placenta in this twin pregnancy was single but a groove separated it into two distinct zones suggesting possible dichorionic pregnancy. The first twin (M) weighed 1·7 kg with oedema of the feet and a slightly webbed neck reminiscent of Turner's syndrome; the second twin (C) weighed 2·1 kg.

(a) Erythrocyte and serum groups: the results of further study of these groups supported the hypothesis of monozygotism. Two Xga group studies showed that these two twins were Xg (a−) like their mother while their father was Xg (a+); but a third check showed "weak activity" for both.

(b) Although drumsticks were present in the two twins, buccal chromatin was negative in M and positive in C.

(c) The palmar and plantar dermatoglyphs were quite similar.

(d) Cytogenetic study made on the blood of the two twins at 10 days, 8 months and 20 months gave the following results: M with Turner's syndrome 28 X0, 65 XX; C (normal) 21 X0, 95 XX.

III. Alongside the above observations it is possible to mention two others which cannot be claimed to be similar since in both cases the karyotypic control of the malformed twin could not be made.

Benirschke and Sullivan (1965a) who reported on the first case examined the survivor of a pair of twins issuing from a monochorionic, diamniotic pregnancy. In the blood of the normal girl, with positive buccal chromatin, of 120 cells 115 had 46 chromosomes and 5 had 45 which, lacking a large metacentric, were considered as X0. The examination of the sister with several deformities and with webbed neck, who died at birth before the advent of cytogenetics, had shown very small ovaries with some tubular formations and only very few primary ova. Various tissues were chromatin negative.

The second case (Shine and Corney, 1966) was that of a pair of monozygotic twins, one normal, the other with several deformities. The abnormal twin was chromatin negative. The erythrocyte and serum groups of these twins were concordant except for the Xg phenotype. In fact, the normal twin was Xg(a+)

and her abnormal sister Xg(a−). The father being Xg(a+) the authors concluded that the latter had not received the paternal X chromosome. However, since autopsy revealed ovaries and histological examination normal ovarian tissue, it must be assumed that the XX line was represented in the gonads and that this abnormal twin was probably X0/XX.

Diplo 21-triplo 21 variety

I. This third variety (Obs. 5) represents monozygotic discordance between two brothers, the first normal, the second with trisomy 21 after a monochorionic and diamniotic pregnancy (Lejeune *et al.*, 1962d; de Wolff *et al.*, 1963).

Examination of these twins born in 1958 confirmed that the first was in all respects normal while the second was a typical trisomic 21, with spherical and small skull, narrow palpebral fissures, oblique superiorly and laterally and with epicanthus, nasal notch, poorly outlined ear lobes without lobules and with hypotrophy and hypotonia. These signs were accompanied by retarded mental development and a systolic murmur linked with probable interventricular communication.

(a) The karyotype of the cultured skin cells gave the following results. Normal twin 44 A + XY; trisomic 21 44 A + XY + 21 and hence free trisomy. Skin mosaicism was not observed in either. The karyotypes of the parents were normal.

(b) Study of the criteria of twinning could not take into account the characters which bear the imprint of trisomy 21. The two twins in fact had fair hair of identical shade but curly in the normal and straight in the trisomic 21. The irises were of the same colour but dotted only in the trisomic 21 with Brushfield spots, more frequent in such patients. They had identical palmar ridges but the trisomic 21 in addition had a transverse palmar fold on the left, and a pseudo t″ triradius on both sides. A mirror image for a pattern in P_{11} on the left in the trisomic 21 and on the right in the normal child completed these discrepancies.

On the other hand, familial study of the erythrocyte, serum and salivary phenotypes demonstrated the identity of the two twins in respect of all the factors studied and that the probability of such a resemblance between dizygotes does not exceed $p = 0{\cdot}000225$. The early death of the trisomic 21 twin prevented a study of the blood karyotype. This research made only in his normal brother when he was about 8 years old showed the presence in 104 cultured cells of 102 44 A XY and two with 44 A XY + 21. A blood mosaicism thus existed here in this normal survivor of a monozygotic diplo 21-triplo 21 pair.

It is interesting to relate this finding to the results of calculation in both these twins of the polymorpho nuclear segmentation index (NSI).

This index, since it is based on the number of lobules for 100 nuclei is lower the more the Arneth formula is shifted to the left. While its normal value is around 300, for trisomic 21's it is usually 270.

Furthermore, the correlation of the Arneth formula between normal twins

TABLE 13.2

Father, Mr. F 44 A, XY	Mother, Mrs. F 44 A, XX	P. F. ♂ / ♂ M. F. 44 A, XY + 21 / 44 A, XY	W. F.	B. F.	Probability of phenotypic concordance
		Erythrocyte phenotypes			
A 1	A 1 B	A 1	A 1	A 1	0·391
MNSs	MNss	MNss	MNSs	MNss	0·250
P 1	P 1	P 1	P 2	P 2	0·625
CCDee	CCDee	CCDee	CCDee	CCDee	0·999
Kp (*a* −), K −	Kp (*a* −), K −	Kp (*a* −), K −	K −	K −	1·000
Lu (*a* +)	Lu (*a* −)	Lu (*a* +)	−	−	0·510
Le (*a* − *b* −)	Le (*a* − *b* −)	Le (*a* − *b* −)	−	−	...
Fy (*a* − *b* +)	Fy (*a* + *b* +)	Fy (*a* − *b* +)	Fy (*a* − *b* +)	Fy (*a* − *b* +)	0·500
Jk (*a* + *b* +)	Jk (*a* + *b* +)	Jk (*a* − *b* +)	Jk (*a* + *b* +)	Jk (*a* + *b* −)	0·375
Xg (*a* +)	Xg (*a* +)	Xg (*a* −)	−	−	0·250
Lef (−)	Lef (−)	Lef (−)	—	−	...
		Serum phenotypes			
Gm (*a* − *b* + *x* − *r* − *e* +)	Gm (*a* + *b* − *x* + *r* + *e* −)	Gm (*a* + *b* + *x* + *r* + *e* +)	Gm (*a* + *b* + *x* −)	Gm (*a* + *b* + *x* −)	1·000
Inv (*a* + *b* + *I* +)	Inv (*a* − *b* + *I* −)	Inv (*a* + *b* + *I* +)	−	−	0·500
Hp (2–1)	Hp (2–2)	Hp (2–1)	Hp (2–1)	Hp (2–2)	0·500
Gc (2—2)	Gc (1–1)	Gc (2–1)	Gc (2–1)	Gc (2–1)	1·000
		Salivary phenotypes			
Se	Se	Se	−	−	0·837
Le	le le	Le	−	−	0·737
					II = 0·000225

is much higher if they are monozygotic ($r = 0{\cdot}802$) than if they are dizygotic ($r = 0{\cdot}419$) (Turpin and Bernyer, 1947a). However, the trisomic 21 member of the pair under consideration was not distinguished from normal by the classical shift to the left in the Arneth formula which usually accompanies his disease (Turpin and Bernyer, *loc. cit*). His index 292 was close to the normal average and almost the same as his non-trisomic brother at 291. This concordance was in line with that which can be expected for monozygotic twins. It indicated that the reciprocal grafts of stem cells in the haematopoietic centres because of early common circulation had probably eliminated the difference which normally exists between trisomic 21 and non-trisomic 21 subjects and between mongol and non-mongol dizygotic twins. The presence in the blood of the normal twin at the age of 8 years of trisomic 21 cells supports this assumption of a stem cell graft.

II. A second preliminary observation on a trisomic 21 boy and a normal boy appeared after a supplementary study to involve a dizygotic pair (Nijenhuis, 1965).

III. A pair of twins one trisomic 21 and the other normal was reported by Dekaban in 1963. Their father and mother aged respectively 30 and 25 years at the time of birth were normal and without karyotypic anomalies. The twins have a younger sister of normal karyotype. The progeny of a maternal cousin included a child with trisomy 21. According to the familial study of the erythrocytic and serum groups the probability of dizygotism was very low, $p = 0{\cdot}00331$. The palmar-plantar dermatoglyphic similarities were compatible with diagnosis of monozygotism. Examination of the blood of the normal twin showed for 30 cells 27 of 44 A + XX karyotype. But the case was complicated by the discovery in the trisomic 21 girl of 48 chromosomes through trisomy G and a small unclassifiable submetacentric neochromosome. Despite the latter the trisomy 21 of this twin did not differ from the classical disease. The author later considering the possibility of monozygotism derived from a trisomic 21 or normal zygote suggested segregation disturbances and structural aberrations which may for both zygotic possibilities explain these karyotypic differences.

But, unlike the preceding observations, no mention is made in this case of cells of the abnormal monozygote in the normal monozygote, or vice versa. We must await confirmation of this example.

The twins whom we have considered have in common the following peculiarities.

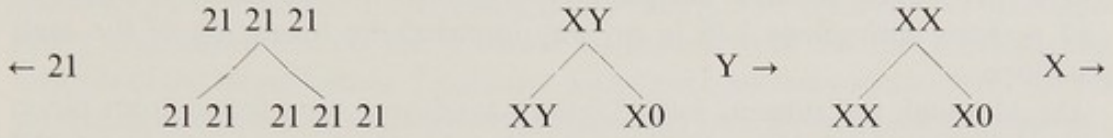
(1) They are pairs of dissimilar subjects: either a normal boy and subject with Turner's sydnrome, or a normal girl and subject with Turner's syndrome, or a normal boy and a trisomic 21 boy.

(2) This dissimilarity is surprising since all the pairs are monozygotes. In addition to the criteria given by the observations reported a further argument supporting monozygotism can be advanced: the difficulty of explaining these pathological twins by dizygotism. We would have to assume for each of them the coincidence of two independent zygotes which are affected in parallel by the same pathological development; chromosomal aberration followed each time by a mosaic very particular in its dissimilar phenotypic consequences: normal subject; pathological subject.

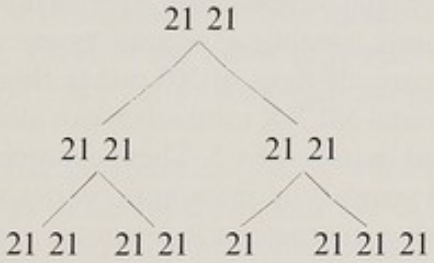
The probability in the hypothesis of a bi-ovular pregnancy of concordance of such a train of events is so small that it indirectly supports the thesis of monozygotism.

(3) Cytogenetic analyses help to explain the phenotypic dissimilarities of these monozygotes by the dominant action of a major clone: normal XY boy and Turner X0 girl; normal XX girl and Turner X0 girl; normal disomic 21 boy and mongol trisomic 21 boy.

(4) An early post-zygotic mitotic disturbance would explain these monozygotisms. It may be pictured by several simple schemes: some involving a normal zygote, the others a pathological one. These schemes presuppose the elimination of a lagging chromosome, a frequent case in Morelle hybrids.



Another hypothesis implies the elimination of an unfavoured cell (lethal)



Another mechanism proposed is asynchronous endoreduplication of a single 21 chromosome in one cell resulting in a trisomic state.

These karyotypic differences which are reflected in phenotypic differences thus justify the term "heterokaryotic monozygotism".

(5) They are more or less complicated by the irregular presence in variable proportion in the blood and skin of the abnormal twin (Turner's syndrome and trisomy 21) of normal cells corresponding to the phenotype of their partners (normal girls or boys), and vice versa.

(a) The blood of the five pairs of twins was checked but one of them (Obs. 5) only in the normal twin, his trisomic 21 brother having died. Normal cells were found (XY or XX or diplo 21) in the majority or almost exclusively in Obs. 1, 3, 4 and 5; pathological cells (X0) in the majority or almost exclusively (Obs. 2).

(b) Skin of both twins: four pairs studied.

The cells corresponded to the phenotype of each and hence were normal or pathological exclusively or in the majority in Obs. 1, 3 and 5 while the cells were of the pathological twin (X0) exclusively or almost so in Obs. 2.

These results give only a partial image of the tissues studied since *in vitro* culture is selective.

In vitro we have noted (Lejeune and Turpin, 1961 b) progressive selection to the advantage of XY cells over X0 in successive passages of the same strain.

It is thus clear that it is not possible to draw from this *in vitro* behaviour any deduction applicable to the *in vivo* course.

(6) These mosaicisms are not very efficient.

(a) Although blood or skin cultures revealed highly varying quantities of X0 or trisomic 21 cells in the twin sisters or brothers of subjects with Turner's syndrome or trisomy 21, the subjects showed normal appearance and behaviour. Further, these cultures in the boy of Obs. 2 revealed virtually only X0 cells. It is thus quite obvious since the role of the Y in masculine determination in the male does not enter this discussion, that the checks (skin and blood) made did not detect XY cells where they exerted their masculinizing action (in the testes) without which the boy would have had a Turner's syndrome with female habitus. These checks only brought to light X0 cells which for reasons of topography and perhaps chronology had in no way impeded the formation of the male phenotype.

(b) Although the subjects with Turner's syndrome were found from blood culture to have only XY cells, for example Obs. No. 1, or a majority of XX cells in Obs. 3 and 4, they were phenotypically of the Turner type. Nevertheless, short stature of 1·45 m or less, webbed neck, epicanthus, possible mental retardation, total ovarian agenesis, absence of secondary sex characters and oedema of the extremities at birth are all signs of X0 rather than of X0/XX.

On the other hand, actual X0/XX mosaicism can alone explain the anatomical characters of the normal twin in Obs. 3. These characters included typical signs of X0 in this child of 3 years 10 months and ovaries with primitive follicles.

X0/XY does not have a characteristic phenotypic expression. The literature mentions various diagnoses: hermaphroditism, masculine pseudo-hermaphroditism, Turner's syndrome with gonadal rudiments (ovarian stroma) and subjects of masculine phenotype with testicular dysgenesis (absence of spermatogenesis). It is thus a question of mosaicisms which hardly betray their presence no doubt because of their localization and the date of their appearance. But they may have immunological consequences. The tolerance of the XY–X0 twins (Obs. 1) for the reciprocal skin grafts does not in fact prove the absence of antigens of histocompatibility linked with the Y chromosome, since the blood mosaicism implies that through the graft there is exchange by virtue of joint circulation of the two embryos in the haematopoietic centres of stem cells from the start of the fourth week (see p. 251). In consequence, even if antigens of histocompatibility existed on the Y chromosome they could not manifest themselves because of "immunological adoption" which these early grafts enjoy.

(7) A final criterion of this monozygotism is fundamental. The mosaicism is not accompanied by a double population. Whenever this has been studied it has not been found. Despite their different karyotypes these cells have the same genetic origin. They produce a mosaicism. If they were of different genetic origin they would produce chimerism.

(8) Heterokaryotypic monozygotism illustrates the often observed coincidence between twinning and disturbances in chromosomal segregation (Klinefelter's syndrome, trisomy 21, Turner's syndrome). These events do not appear from familial twinning or even individual coincidences to be totally independent.

However, a systematic study of the nuclear chromatin of cells adhering to the amnions detached from the placentas of more than 500 pairs of twins born in the last 2 years in Birmingham (Edwards *et al.*, 1966) has not indicated anomalies suggestive of the above coincidence.

(9) The facts revealed by the individual gonado-somatic dysgenesis of chromosome origin may be used to interpret certain features of these pathological monozygotisms.

The future of the gonads depends on their chromosome formula. The majority XY or XX clones determine the masculine or feminine orientation; the X0 major clone determines gonadal agenesis, Müllerian non-hormonal development and feminine phenotype equivalent in humans to the "Jost" effect produced by early embryonic castration in the rabbit. The future of the sex characters depends more on the gonadal hormones than on the chromosome formula of the target tissues. Treatment with oestrogens may stimulate in the X0 subject development of the skeleton, breasts and hair growth providing the target tissues are not too rudimentary.

Heterokaryotic monozygotism with mosaicism differs in its fundamental features from dizygotism with chimerism. This chimerism is very rare. Since the first case (Dunsford *et al.*, 1953) to our knowledge only six have been reported. Since then, the study of 77 twin pairs (Race and Sanger, 1956) did not reveal a double population.

Study of 409 dichorionic pregnancies with a coalescent placenta (Uchida *et al.*, 1964), 238 twinnings of identical sex and 171 of different sexes, gave no indication of interplacental vascular communications.

Another inquiry concerning 500 placentas in dichorionic twinnings (Cameron *et al.*, 1966, unpublished) did not reveal anastomoses except in one case and without appreciable consequences as shown by close study of the erythrocyte and serum antigens of both twins.

The following features according to the rare evidence published (karyotypic analysis has been reported only twice) distinguish chimerism of dizygotic twins from the mosaicism of monozygotes:

(1) Each pair is composed of normal subjects without signs of intersex.

(2) These pairs are all dizygotic.

(3) All the observations refer to a double blood population.

(a) Most often the majority clone of the double population corresponds to the genotype of the recipient, the minority clone to the genotype of the donor.

If the recipient is an XY boy and the donor an XX girl the double erythrocyte population will thus be mostly composed of XY cells. This type of chimerism would be that of marmosets. In this variety of monkeys, 87 per cent of the births are twins, mostly dizygotes. Study of the bone marrow and the testes in three such animals (Benirschke *et al.*, 1963) uncovered a chimerism. The majority clone in the bone marrow corresponded to the genotype of the recipient. Despite numerous interplacental anastamoses, free martinism is said not to exist in marmosets.

(b) The only report on such a case in humans (Chown *et al.*, 1963; Uchida, 1964) mentions chimerism of the same composition in both zygotes, a normal

girl and a normal boy with about two-thirds of the cells XX, group 0 and one third XY, group A. This type of chimerism is that of bovines.

(4) The reciprocal skin graft test between partners of a dizygotic pair has confirmed in rare cases of chimerism the tolerance expected.

(5) The human dizygotic chimeras may be unilateral and ephemeral. We have an example (Turpin, Salmon and Cruveiller, 1959b) 127 days after birth in the twin brother of a girl. The double population had only 5 per cent of the cells of the sister. These foreign cells had disappeared at the sixth month. This reject implied an immunological process which could not be confirmed by investigations of antibodies to erythrocyte, platelet and leukocyte antigens. Chimerism which appears in man is a very rare complication of dizygotism and thus differs in its fundamental features from the mosaicism of dissimilar monozygotes.

Two processes may be invoked to explain this mosaicism associated with monozygotism—one accompanying the separation of the two twin rudiments, the other accompanying the establishment of the embryo-chorionic circulation.

(1) The first process may accompany the relatively early cleavage of the embryonic bud. According to the presumed mechanism (see above) this bud by virtue of the anomaly of chromosomal segregation at formation of the first blastomere, may consist of two clones of different karyotypes, XY and X0, for example (Obs. 1 and 2). As the embryonic bud develops the cell movements modify the initial relations between the blastomeres and shift the various regions of the embryo in relation to each other. Depending on the moment of separation of the two twin rudiments and the orientation of the plane of cleavage, this cleavage should occur without or with the more or less wide incorporation of cells from one of the rudiments in the other, and vice versa. However, the observations so far reported all show that the conditions of cleavage of the embryonic bud do not entail a mosaicism capable of achieving between these monozygotes the phenotypic similarity which might be expected. Each retains the phenotype of its clone of origin: XY, XX or diplo 21 for normal subjects; X0 or trisomy 21 for pathological subjects. It must therefore be assumed that the circumstances of development of the embryonic bud and then of cleavage incorporate only a few or no cells from one of the partners in the other, and vice versa.

It is possible that the phenomenon of aggregation of cells of the same line brought into play by certain conditions of culture operate here (Moskowitz, 1964). These aggregates reflect an intercellular adhesive capacity which persists when the cells divide. They may grow and acquire their own morphological features depending on the line of origin. The structure of an aggregate depends on the conditions in which it develops and the powers of the cells which compose it. If an XY zygote gives as a result of an accident in chromosomal segregation an XY blastomere and an X0 blastomere it is possible that the XY clonal cells will tend to form an XY aggregate and the X0 cells an X0 aggregate. The result would be a kind of bipartite embryonic bud lending itself, in the event of fairly early cleavage, to almost perfect separation between the two aggregates with different chromosome formulae, the origin of the heterokaryotic monozygotic pair.

(2) A plausible explanation of this mosaicism is provided by the development of embryo-chorionic circulation common to both twins and set up at the beginning of the fourth week following conception.

This circulation, in fact, opens the way to stem cell grafts from one of the twins to his partner, and vice versa. A key role is no doubt played by the lymphocyte, a young cell capable of developing in diverse ways, and by mobile or readily mobilizable cells of the histiocytic type (reticulo-endothelial system).

Such transfusion may be expected to give an unequal cell distribution not only because one of the twins is more transfuser than transfusee, and vice versa, but also because of the possible selective influence of the different media in which these cell grafts are induced to develop.

These mosaicisms because they are relatively late and because they probably do not affect the primary centres of embryogenesis may all have little effect on the phenotype which remains identical, to judge by the evidence so far gathered, with the clone of origin.

A further remark may be made on heterokaryotic XY–X0 monozygotism. The first X0 Turner twin, a sister of an XY boy (Obs. 1), was free of all signs of masculinization suggestive of free martinism. The latter according to recent evidence (Ohno *et al.*, 1962d; Fecheimer *et al.*, 1963) is thought to derive from a double mechanism. One, a cellular mechanism, transfusion of masculine cells, followed by gonadal grafts chiefly responsible for the masculinization of the internal genitals. The other, hormonal, transfusion of androgens of the male twin acting mainly on the external genitals.

It is not surprising that in absence of gonads an intersex state with ovotestis, genuine human free martinism, cannot develop. But it is surprising to find in the external genital organs of this X0 none of the effects of the XY cells and androgen hormones transfused by his XY twin. Ephemeral intra-uterine virilization cannot be invoked since no sign of feminine pseudo-hermaphroditism is noted at birth.

Nor does this indifference to the substances inducing Wolffian stimulation and Müllerian inhibition secreted by the XY foetus appear to be due to the abnormal X0 chromosome constitution of the competent tissues.

We have, indeed, seen one of our X0 patients who was hypotrophic, respond to an anabolizing protein with androgenic powers, like an XX girl. The frequency of free martinism in bovines contrasts with its rarity if not absence, in particular, in man and the marmoset. In man interplacental vascular anastomoses are no doubt very rare between dizygotes and are perhaps too late. But in the marmoset dizygotism with interplacental anastomoses is very common. It may therefore be asked whether free martinism is the result of hormonal or cellular transfusion or both and if certain conditions such as sensitive periods of embryogenesis, dose and lifetime of the messenger products do not take an essential part in its appearance.

Heterokaryotic monozygotism while allowing important deductions also poses problems the solution of which largely depends on a deeper knowledge of the relations of placental physiopathology and embryogenesis.

CHAPTER 14

Mechanism and Effects of Numerical Gonosomal Aberrations

MECHANISM

I. Numerical aberrations without mosaicism

The problem of the origin and mechanism of numerical chromosome aberrations arose as soon as trisomy 21 was discovered. Since this human trisomy compatible with life, and even reproduction, had no equivalent in other mammals it was necessary to look for analogies among lower animal and plant species.

To this end attempts were made to inculpate non-disjunctions during gametogenesis (meiotic) or the development of the zygote (mitotic) and chromosome losses.

A. Abnormal gametogenesis

(Non-disjunction of meiotic origin)

Experimental findings

An explanation provided by the genetics of *Drosophila*, that of an aberration called "non-disjunction", was soon invoked. It offered 40 years ago an interpretation of facts very close to those now discovered by human cytogenetics. In mentioning this analogy we at the same time pay tribute to the person who was to formulate and verify the hypothesis, C. B. Bridges. Studying the transmission of an X linked character in the normal state (red eyes), and in the recessive state "white eyes", this pupil of Morgan found, according to the theory of the latter, that the progeny of "white eye" female and "red eye" male pairs nearly all consisted of white-eyed hemizygous males and red-eyed heterozygous females.

But exceptional fruit flies or "patrocline" males with red eyes and "matrocline" females with white eyes appeared in the proportion of 1 in 2000 to 3000. To explain their appearance, Bridges pictured an accident during the complex mechanism of meiosis which governs formation of the gametes. The formation from oogonia or spermatogonia with $2n$ chromosomes (diploid) of ovules or spermatozoids with n chromosomes (haploids), presupposes the faultless unfolding of the various phases of a long and complicated process essential to the preservation of the hereditary legacy of the species.

(a) He claimed that this accident occurs in the first so-called reductional meiotic division (heterotypic) since it enables the oocyte (or the spermatocyte) of the first order to distribute its $2n$ chromosomes into two equivalent sets of n, one for each of the two oocytes (or spermatocytes) of the second order. In fact, division of the first order oocyte gives the oocyte of the second order and a first polar body.

At the zygotenic subphase of prophase I (synapsis) the two X homologues pair off like the other homologous chromosomes, the paternal and maternal. They synapse along their length. This conjugation of two univalents forms a bivalent which at the following subphase, pachytene, shortens and thickens. Then, in the diplotene subphase, each of the univalents forming it doubles into two chromatids with possibilities of recombination and the bivalent becomes a tetrad. At the last stage of the prophase (diakinesis) the nuclear membrane disappears.

At metaphase I the bivalents arrange themselves along the equator of the spindle, the chromatids symmetrical with respect to the equatorial plane. Then at anaphase I fundamental segregation puts an end to the pairing of the two univalents. Each of them formed from its two chromatids still united by the undivided centromere and guided by the spindle fibre takes part at one pole of the spindle in formation of one of the two son nuclei thus equipped with n chromosomes.

(b) The second meiotic division known as equational (homeotypic) enables the oocyte (or the spermatocyte) of the second order to distribute its n chromosomes which have become $2n$ chromatids between its two daughter cells. It therefore involves disjunction at anaphase II which enables each gamete to receive n chromatids, that is, a complete set of chromosomes peculiar to the species but each as a single copy. Each ovule is equipped with an X chromosome, each spermatozoid with a Y or an X chromosome.

Meiosis therefore entails two cell divisions for a single chromosome duplication. It requires two disjunctions and leads from a germinal cell with $2n$ chromosomes to gametes with n chromosomes. It is strictly equivalent for both sexes but whereas spermatogonia give four spermatozoids, oogonia give one ovule and three polar globules.

On the other hand, mitosis entails for single chromosome duplication only one cell division. It requires only one disjunction and distributes in each of the daughter cells $2n$ chromosomes, that is, a complete set of chromosomes peculiar to the species, one of paternal origin, the other maternal origin, in duplicate.

According to the hypothesis of Bridges, assuming that at the first meiotic division the two X univalents sometimes do not separate, the second order oocytes will receive two X univalents whereas others will receive none. Thus, after the following normal equational division, certain ovules will have 2 X and others none at all. The fertilization of these abnormal ovules by normal spermatozoids will give in theory four types of zygote: XXY, X0, XXX and Y0. Cytological examination enabled Bridges in 1914 to verify the soundness of his hypothesis. Apart from the Y2A fruit fly presumed to be non-viable, the three other types were found: the 3X2A fruit fly hypogonadal and sterile with

malformed eyes and wings usually not going beyond the larval or pupal stage; the XXY2A fertile female fly; and the X2A sterile male.

The XXY fly was a female with white eyes having therefore received the two maternal X chromosomes; the X0 fly was a male with red eyes having received the paternal X chromosome.

This brilliant demonstration was repeated during study of the haplo IV fruit fly and led Bridges (1921) to the discovery of his reciprocal type, the triplo IV fly.

Transposition to humans

These experimental findings do not provide absolute proof of simple human transpositions. As far as we know, no definite cytological evidence of meiotic non-disjunction in man has yet been provided.

However, a defect in pachytenic pairing of the X and the Y chromosomes at prophase I has been noted, the X and Y being situated apart in a highly "heteropycnotic" special sex vesicle" (Sachs, 1955). This defect in XY pairing has been reported by other authors for metaphase I (Ford and Hamerton, 1956c).

Despite the absence of cytogenetic proof, meiotic non-disjunctions provide explanations of the appearance of human aneuploidies plausible enough to justify further discussion.

Familial study of X chromosome-linked recessive diseases found among parents and sibs of subjects with Turner's or Klinefelter's syndromes may provide an argument in support of a simple non-disjunction during gametogenesis and an indication of paternal or maternal origin.

Daltonism has been a real aid in such study. This dyschromatopsy, recessive in relation to the normal colour sense, may be detected in a simple and rapid manner thanks to the Ishihara tables. In addition, its various subgroups may be distinguished by examination with the anomaloscope; protanopy, blindness for red recessive in relation to the protanomaly, weakness for red; deuteranopy, blindness for green recessive in relation to the deuteranomaly, weakness for green.

The incidence of daltonism is 8 in 100 in the Indo-European races in males and 0·43–0·44 in females (Waaler, 1927). The difference between this latter figure 0·44 and the theoretical frequency in females $8\% \times 8\% = 0{\cdot}64$ per 100 is explicable by the bilocular theory of Waaler–Franceschetti. According to this theory which has recently been reinforced by strong familial arguments (Franceschetti, 1962), the genes on which the two main forms of daltonism depend, the protan group, on the one hand (protanopy and protanomaly), and the deutan group, on the other (deuteranopy and deuteranomaly), are not alleles but are situated on different loci of the X chromosome. Thus, a female possessing a gene for the protan group on one of the X chromosomes, and a gene for the deutan group on the other, is not daltonian since each of these recessive genes is dominated by its normal allele situated on the other chromosome.

Simple non-disjunction

In line with usage, we shall employ the current expression "non-disjunction" serving to designate an anomaly of chromosome or chromatid distribution whatever its mechanism, viz. anomalies in pairing, defect in chromosomal segregation, non-disjunction of chromatids.

(1) Turner's X0 syndrome

From study of X-linked characters it is therefore possible, in principle, to establish the maternal or paternal origin of the X chromosome in X0 subjects. This study offers a means of identifying the gamete deprived of X by abnormal cell division. An ovule without X is necessarily at the origin of an X0 subject with normal vision born to a colour-blind homozygous mother and a father with normal vision; a spermatozoid without X if the X0 subject born to the same pair is colour-blind.

(a) The maternal origin of the X chromosome is now well established in subjects with Turner's X0 syndrome and without evidence of mosaicism. It has been demonstrated by study of X-linked characters.

Colour blindness has guided this research (Polani, 1961d; Lindsten *et al.*, 1963b). Even before cytogenetic studies it was invoked as proof of the maternal origin of the X chromosome in X0 subjects with a major defect in red–green perception, and born to a normal father and mother presumed to be heterozygous (Lenz, 1957; Stewart, 1959a; Bishop *et al.*, 1959). Cytogenetics has increased the number of demonstrative observations.

Deficit in glucose-6-phosphate dehydrogenase (G-6-PD) has also served as a criterion (Gartler *et al.*, 1962b; Adam *et al.*, 1963). The X0 subject observed by Gartler *et al.* had inherited deficiency of G-6-PD from his mother. If the aberration was of gametic origin the spermatozoid must have been the gamete unbalanced through absence of an X chromosome.

The Xg blood group enabled Lindsten *et al.*, (1963b) to establish the maternal origin of the X chromosome in 20 X0 subjects.

Finally, although this is a matter of post-zygotic Turner's syndrome, new proof of maternal origin is afforded by the observations of Turpin *et al.* (1961e) of an X0 girl with normal monozygotic XY twin brother. The X chromosome must have a maternal origin since these two twins came in all likelihood from an XY zygote (cf. Chapter 13).

It seems that in the future these criteria of identification will be enriched by other X-linked recessive morbid states. Before the advent of cytogenetics, Duchenne's muscular dystrophy provided Walton (1955 and 1956) occasion for such a study. The same may be true of Hirschprung's disease (Hayward and Cameron, 1961).

(b) The paternal origin of the X chromosome of X0 subjects is less often demonstrable so that this origin has been the subject of controversy (Muldal, 1962). Some point out in this connection that the X0 mouse takes its X from its mother rather than from its father (Welshons and Russell, 1959). The colour-

blindness criterion has, of course, been used (Polani, 1961 d). Instances of colour-blind X0 girls born to a colour-blind father and normal mother (Frey, 1961; Lindsten *et al.*, 1963b) no doubt provide a good case for the paternal origin of the X chromosome but as the authors themselves admit, do not always allow one to eliminate the possibility of a heterozygous mother.

The Xg blood group (Sanger *et al.*, 1962a) has made it possible to tackle this study with greater precision.

In a first family, the paternal origin of the X chromosome in the X0 subject was established, the X0 being Xg (a+), father Xg (a+) and mother Xg (a−). In the second family the results suggest the same interpretation, the X0 subject being Xg (a+), the mother Xg (a−), while the father could not be examined.

In a total of 56 families analysed, that is, father, mother and X0 child, the X chromosome of the child was in one paternal, in 20 maternal and in 35 of undetermined origin. These results therefore show that the origin of the X chromosome in Turner's X0 syndrome is not solely maternal but can also be paternal. They do not yet permit a calculation of the relative frequency. Finally, other observations are based on the double criterion of dyschromatopsy and the Xg group. One is that of an Xg (a−) Turner's syndrome with colour blindness (deuteranopy) and also the Xg (a−) father, while the Xg (a+) mother, sister and brother were not colour-blind (Almquist).

Another familial observation (Turpin *et al.*, 1964b) is also just as revealing: a colour-blind (protanopy) subject with Turner's syndrome and also her protanopic Xg (a−) father. The brother of the latter was also protanopic. There was absence of dyschromatopsy in the Xg (a+) mother, the two Xg (a+) brothers and the two Xg (a+) sisters of the subject with Turner's syndrome, with absence of known dyschromatopsy in the maternal family.

These facts render very unlikely maternal heterozygotism both for recessive characters (colour blindness) and Xg (a−). They are very suggestive of the paternal origin of the X chromosome in this case of Turner's syndrome: probability of the order of 25,000 against 1, certainly higher than 3000 to 1 (Turpin *et al.*, 1965b).

(2) Klinefelter's XXY syndrome

The presumed origin of the responsible non-disjunction was at first based on the criterion of colour blindness, then on that of the Xg groups.

The possibility of disturbance in spermatogenesis may be considered if a subject with XXY Klinefelter's syndrome possesses like his father a normal vision, while his mother is colour-blind.

On the other hand, disturbances in oogenesis may be considered when the XXY patient is colour-blind while his father is not and his mother is.

Of three cases of Klinefelter's syndrome and colour-blindness, two were deuteranomalous and one protanopic (Nowakowski *et al.*, 1959). No karyotypic study was made in these chromatin-positive subjects.

One of the subjects with deuteranomaly had been born to a normal father and deuteranomalous mother. In all likelihood he originated from an XX ovule through non-disjunction at oogenesis and from a normal Y spermatozoid.

The other deuteranomalous and the protanopic subjects were born to a father and mother with normal vision.

It may be assumed that the latent disturbance in the heterozygous mother became manifest for an unknown reason in the heterozygous sons born after fertilization of an X ovule carrying the blemish by an XY spermatozoid through paternal non-disjunction. Leaving aside this unlikely hypothesis two others can be considered. They, in fact, both account for the appearance in the progeny of a heterozygous female carrier of an X chromosome-linked recessive gene, allele of deuteranomaly or protanopy, of a homozygous XXY son bringing into the open the latent dyschromatopsy in the mother.

(a) The first hypothesis rests on a mechanism for which *Drosophila* gives many examples. It firstly assumes a recombination (crossing over) at the diplotene subphase between one of the chromatids of the X chromosome carrying the allele A and a chromatid of the homologous X carrying an a. If this recombination between the centromere and the locus of A is followed by non-disjunction at anaphase I, ovules AA or aa may result from the second meiotic division. Stern (1959c), recalling this pattern, showed that it is compatible with the facts. Reservation must however be made for the small number of observations.

The frequency of homozygotism brought about by this mechanism depends on the distance between the centromere and the A locus. If, as he says, this distance is so great that the recombination is free, we may expect from random sorting, a frequency of 16·7 per cent. But since the frequency of females heterozygous for one or the other X chromosome-linked recessive alleles of colour blindness is put at 15 per cent, it may be deduced that one in six of their sons with Klinefelter's syndrome will be colour-blind, that is, about 2·5 per 100 ($\frac{1}{6} \times 15$ per cent) in all the Klinefelter's syndromes which result from this mechanism.

To this 2·5 we must add subjects who may come from homozygous mothers and hence also be affected, that is, about 0·5 per 100 and those who may derive from an XY non-disjunction of affected fathers married to a woman homozygous or heterozygous for the stigma, that is, again 0·5 per 100.

Since the condition varies with the relative proportion of those who owe the Klinefelter's syndrome to maternal non-disjunction in relation to those who owe it to paternal non-disjunction, it may be estimated that colour blindness among these patients must vary between 0·5 in 100 and 2·5 + 0·5 = 3 per 100 (Stern, 1959c).

Two inquiries (Polani *et al.*, 1958; Nowakowski *et al.*, 1959) brought to light three patients with Klinefelter's syndrome affected by deuteranomaly or protanopy in 106 of these patients or 2·8 per 100. The close relation between the supposed and recorded facts is undeniable but its value is reduced by much uncertainty.

In the absence of cytogenetic examination these 106 patients in fact are presumed to be XXY since they are chromatin-positive. The three subjects with colour blindness were detected among 34 patients in one of the inquiries (Nowrowski *et al.*, 1959) while the 72 of the other (Polani *et al.*, 1958) were not colour-blind. Some of the numerical estimates are very approximate.

New inquiries with karyotypic analyses are therefore necessary to make a more factual study of Stern's interesting arguments.

(b) The second hypothesis is that of non-disjunction at anaphase II. Since the number of heterozygous mothers is put at 15 per 100 and that of homozygous 0·5 per 100 we may expect for the former 7·5 per 100 and for the latter 0·5 per 100 colour-blind subjects in Klinefelter's syndrome. Stewart (1959a) points out that this figure of 8 per 100 does not take into account the possibilities of recombination, and discussing the chances of recombination prior to this non-disjunction at the second meiotic division he shows that we may expect from this dual mechanism 3 per 100 recessive homozygotes including 2·5 per 100 born to heterozygous mothers ($\frac{1}{6} \times 15$ per cent) and 0·5 per 100 born to homozygous mothers.

The expected frequency of Klinefelter's syndrome with colour blindness resulting from an anomaly of the second meiotic division could be 3 to 8 per 100.

To add greater precision to these discussions of unquestionable interest we must have a clear idea of the chances of homozygotism for the colour blindness allele in man. We need to have at our disposal results of wider inquiries and diagnoses supplemented by karyotypic analyses.

(c) While the colour blindness marker gene of the X chromosome allows us to discuss various mechanisms of the human XXY subjects through disturbance in oogenesis, five X marker genes in the mouse show that an exceptional variety of XXY may result from non-disjunction at the first meiotic division of a normal male (Russell, 1961a). If the X chromosomes depending on their maternal or paternal origin are distinguished by m and p, the crossings made have shown that X^mX^pY to be much less frequent than the type X^m0. On the other hand, no case of X^mX^mY or $0X^p$ has appeared. No evidence of XX non-disjunction at the first meiotic division in the female mouse has yet been obtained.

(d) Study of the Xg blood group in four families (Frøland, 1963b) gave interesting results.

The first XXY subject being Xg (a+) could have only received an Xg (a+) gene from the father, the mother being Xg (a−) and the sister Xg (a+). This subject would be the human equivalent of the XXY mouse mentioned above and shows that XXY may result from non-disjunction during spermatogenesis (first meiotic division).

The three other families give examples of XXY subjects who are Xg (a−) and who could not have received the paternal Xg (a+). In one of these families the Klinefelter's syndrome was present in two twins presumed to be monozygotic. The non-disjunction responsible for the allocation of two maternal X chromosomes may have been both pro- and meta-zygotic.

(3) Schemes of simple non-disjunction

Figure 14.1 schematizes the consequences of a non-disjunction occurring at anaphase I or at anaphase II in oogenesis or spermatogenesis.

Assuming that each of the non-disjunctions is equiprobable, the non-disjunction during oogenesis will result in the zygotes XXY, X0, XXX and Y0 in

equal proportions. The Y0 type has never yet been encountered; it is no doubt non-viable as is its *Drosophila* counterpart.

Non-disjunction during spermatogenesis will result in zygotes X0, XXY, XXX, XYY but in unequal proportions, namely, four X0, two XXY, one XXX and one XYY, respectively. These types are all known.

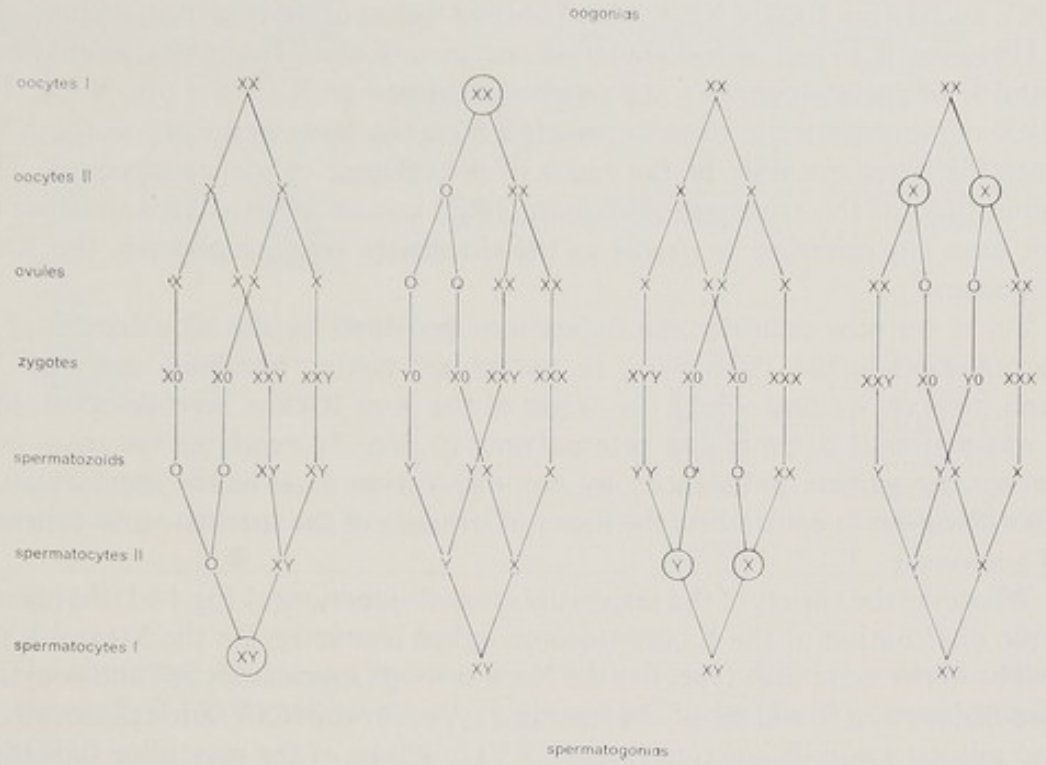


FIG. 14.1. Meiotic non-disjunction of the X chromosome. Anaphase I or II.

If we assume an equal frequency of the non-disjunctional varieties in Fig. 14.1, five types of zygotes distribute themselves in the following proportions: six X0, four XXY, three XXX, two Y0 and one XYY.

To test the value of the theory of meiotic non-disjunction it is therefore necessary to compare these proportions with results of inquiries into the distribution of gonosomal aberrations among the newborn.

According to the chromatin-body criterion the frequency of the XXY anomaly including mosaics would be 2·06 per 1000; that of the XXX 1·2 per 1000; that of the X0 0·37 per 1000. These results are disconcerting. The proportion of X0 is not greater than 0·37 per 1000 although assuming that the frequency of the non-disjunction varieties is the same it ought to be equal to about the sum of the XXY and XXX or 3·26 per 1000.

To explain this X0 deficit several hypotheses may be advanced.

The first is that of considerable mortality of X0 zygotes. We believe it to be valid, despite the good viability in general of X0 children. The latter are perhaps only the survivors of a natural selection which only allows 10–15 per cent survival of these fragile eggs. Study of mortality *in utero* of chromosomal aberrations would no doubt be highly instructive (p. 165).

The second supposes that the spermatozoid minus gonosome is greatly reduced by this anomaly (Bateman, 1962). It may be incapable of fertilization or even of life. The ovule, on the other hand, would be better adapted to this loss. According to this hypothesis a maternal non-disjunction is responsible for 0·37 per 1000 of X0 and an equivalent number of XXY and XXX anomalies. Consequently, taking into account the results of the above inquiry, 1·7 per 1000 of XXY and 0·8 per 1000 of XXX would be the product of paternal non-disjunction.

However, if, in fact, non-disjunction occurs with equal frequency, at anaphase I and II of spermatogenesis, the result should be two XXY for one XXX. The XXX of paternal origin must in principle have the same frequency as the XYY since the latter can only be the result of disturbance in spermatogenesis. This implication of the argument (Bateman, 1962) cannot be used here since we do not have any criterion as simple as the chromatin body to estimate the XYY frequency.

But if we now consider the indications provided by the distribution of X chromosome-linked characters, in particular, colour blindness and the Xg gene marker, we find where the origin of the X of X0 has been detected, that it was maternal 20 times and paternal once (p. 266). In nearly all the cases now known the gamete unbalanced by non-disjunction must be the spermatozoid. This observation contradicts the thesis of lethality of the spermatozoid deprived of gonosome.

Whatever the variety of the responsible non-disjunction (cf. Fig. 14.1) the phenotypic distribution of the X chromosome-linked characters for the X0 and XYY will be of the masculine type. For the XXY through spermatocytic I and oocytic I non-disjunction it will be of the feminine type; for the XXY through oocytic II and mitotic I non-disjunction (zygote XY) it will be of the masculine type (Saldanha, 1962). For the XXX subjects it will be of the feminine type except for the XXX which results from an oocytic I non-disjunction. This latter variety in fact implies in its recessive form encounter of three homologous chromosomes each bearing the recessive gene; the distribution calculated (Saldanha, 1962) would be 94 per cent Xg (a+) and 6 per cent Xg (a−).

Finally, a third explanation based on solid arguments is that of X0 through anomaly of the first zygotic mitoses. This possibility is considered in the following section: abnormal embryogenesis.

The particular case of the progeny of XXX mothers was considered in studying this morbid type.

To explain other types of gonosomal aneuploidy which mere non-disjunction cannot bring about, more complex disturbances in gametogenesis have been evoked. These are associated non-disjunctions. It is also important to note the theoretical possibility of loss of chromosome (p. 272).

(4) Schemes of associated non-disjunctions

There may be a random encounter of a spermatozoid and an ovule both abnormal through disturbance in one or the other of the two meiotic divisions. The possibility of the meiotic anomaly affecting one or other of the two divisions cannot be dismissed. Segregation of the homologous chromosomes does not

occur during the first division; nor does disjunction of the chromatids occur at the second division or it inolves only two chromatids.

Associated non-disjunctions. Normal or abnormal gametes through disturbance of 1st, 2nd or both meiotic divisions

Spermatozoids	Ovules	21 theoretical zygotic combinations		
		Normal	Abnormal	
0	0	(2)	Known	Unknown
X	X			
XX	XX	XX and XY	(10)	(9)
Y	XXX		X0	0
XY	XXXX		XXX	Y0
XXY			XXXX	YY
YY			XXXXX	XXXXYY
XYY			XXY	XXXXXY
XXYY			XXXY	XXXXXYY
			XYY	XXXXXX
			XXYY	XXXXXXY
			XXXXY	XXXXXXYY
			XXXYY	

The abnormal ovules and spermatozoids which result from this double meiotic anomaly if fertile will give birth in theory to a large variety of aneuploidal zygotes.

The ovules may in fact be fertilized by normal spermatozoids or abnormal ones through disturbance in the first or second or both meiotic divisions.

The spermatozoids likewise may fertilize normal ovules or abnormal ones through the same meiotic disturbances.

All these theoretical possibilities lead us to imagine encounters of ovules 0, X, XX, XXX, XXXX and of spermatozoids 0, X, XX, Y, XY, XXY, YY, XYY, XXYY. The result would be 45 theoretical fertilizations giving 21 zygotic varieties: some normal XX and XY; others known pathological ones X0, XXX, XXXX, XXXXX, XXY, XXXY, XYY, XXYY, XXXXY, XXXYY; the others unknown pathological ones. The latter group no doubt includes a large number of forms which cannot be realized because of gametic impotence; lethal forms through zygotic impotence, 0Y, for example, and agonosomal 00; exceptional and still undetected forms implying the unlikely coincidence of gametes themselves exceptional.

An estimation of the probability of each of these different combinations has been proposed (Twiesselmann *et al.*, 1963). This is only tentative in view of our ignorance of the causes and mechanism of human meiotic anomalies. For example, there is nothing to show complete independence of the anomalies of the first and second division, and the estimates were made without it being possible to take into account this possible interaction.

B. Abnormal embryogenesis

A large volume of pathological evidence plus experimental arguments suggests that certain chromosome aberrations originate from early anomalies in embryogenesis. These anomalies are mitotic non-disjunctions and chromosome losses.

1. Mitotic non-disjunction

Non-disjunction at the first cleavages, even possibly the very first cleavage of a normal or abnormal zygote, may produce in theory various types of blastomeres, which may help to explain the origin of certain types of numerical aberration, XXYY and XXXX, for example, without resorting to unlikely encounter of meiotic non-disjunctions.

(a) If only one of the first two blastomeres is viable, different mostly pathological chromosome types are conceivable, for which Fig. 14.2 gives some examples.

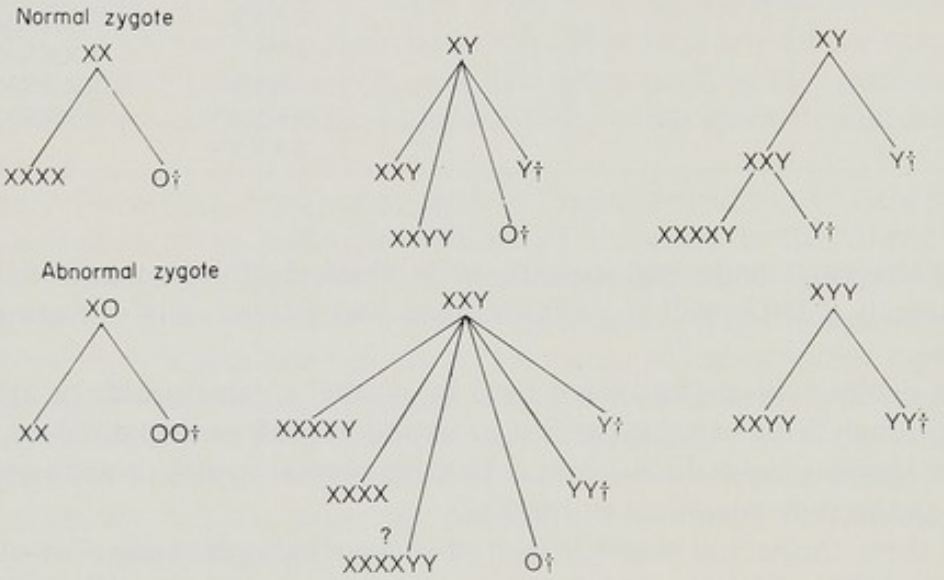


FIG. 14.2. Examples of abnormal segregation of the X chromosome on cleavage of the first blastomeres.

(b) If the two blastomeres are viable, a mosaic will result from their parallel development (see above). But if one of these two lines is favoured by selective factors it may gain the upper hand to the point of eliminating the other. Thus, an initial mosaic process would taper out. Such elimination of cells has been observed in haematopoietic tissues of irradiated mice (Ford *et al.*, 1959d) and also in the bone marrow and irradiated testicles of a variety of marsupials *(Potorous tridactylous)* (Sharman, 1959).

2. Chromosome losses

The loss of a "lagging" chromosome is a possibility exemplified by certain plant hybrids among others.

This chromosome does not move at anaphase. It remains between the two poles without taking part in formation of the two daughter cells. These chromosomes are often found in hybrid species (Morelle hybrids) or in cases of structural chromosome aberrations (translocations). The fact that this phenomenon exists in man is suggested by observations of heterokaryotic monozygotism. It has been established experimentally by irradiation of the female mouse.

This irradiation at different intervals immediately after fertilization may cause elimination of a sex chromosome, increasing the chances of appearance of X0 animals: 7·2 per cent in one group, 5·1 in a second, 2·8 in a third, as against a mean value of 1·3 per cent among the controls (Russell, 1961a).

Exposure of the egg to ionizing radiations immediately after fertilization is said to be particularly damaging. The chromosomes of the mouse are thought to be very labile and highly radiosensitive between fertilization and the first cleavage of the zygote. X0 animals are thought to result more often from a process after fertilization than a disturbance in gametogenesis.

II. Numerical aberrations with mosaicism

The term "mosaic" was used by Charles Naudin when he published in 1858 and 1863 the results of crosses between different plant races.

These crosses enabled him to establish beyond doubt disjunction in the second generation of the parent characters temporarily united in the hybrid. But, in addition, they led him to note that in the first generation some hybrids are distinguished from the general intermediate type by the presence side by side of different parental traits. Hybrids of *Datura laevis* with smooth capsules × *Datura stramonium* with spiny capsules bore, for example, on the same fruit, both smooth and spiny characters.

From the appearance of these "mosaics" he drew fundamental deductions which clarified the mechanism of heredity. As Louis Blaringhem remarked, the conclusions in the paper by Naudin "are the first clear exposition of the principle of mosaic heredity which dominates all research into heredity and includes as a particular case alternating or Mendelian heredity". This initial description was followed by many other examples of mosaicism even microscopic (crystals of calcium oxalate isolated or grouped in hummocks).

Since 1959, human cytogenetic research has led to the discovery of subjects formed by the assembly of two or more cell types with different chromosome formulae. These subjects have been termed "mosaic" although they are not characterized as are Naudin hybrids by a variegated aspect. Their mosaicism is also different from that of the heterozygous female mouse the markings of whose coat are explained in the hypothesis of Lyon (p. 283) by chance inactivation of one or other of the two X chromosomes. The karyotypic reality of gonosomal mosaics the idea of which was suggested by study of the chromatin body (Danon and Sachs, 1957) was established for the XX/XXY variety by Ford *et al.* (1959b).

These mosaicisms detectable by cytogenetics may in theory have three differ-

ent origins depending on the time of their formation, compatible with a process which precedes, accompanies or follows formation of the zygote.

(a) The first alternative was invoked for an observation of XX/XY hermaphroditism. This chromosome mosaicism coincided with a mosaicism of the erythrocyte and serum phenotypes (Gartler *et al.*, 1962; Grouchy *et al.*, 1964; Zuelzer *et al.*, 1964). It was therefore explicable in terms of double fertilization of a binuclear ovule. Such a mosaicism of gametic origin (binuclear ovule, polyspermy) may be an example of mosaicism preceding formation of a zygote.

(b) The second alternative is that of fusion of two zygotes. The observations which have just been mentioned are also amenable to this explanation. The hermaphrodite would then result from fusion of an XX zygote and XY zygote before any cleavage. Its formation would coincide with that of the zygote XX/XY itself.

(c) The third alternative is that which appears to be the most common, involving segregation anomaly through chromosome non-disjunction or loss of a lagging chromosome (see p. 273). If this process occurs at the first cleavage of the normal or abnormal zygote, a mosaic can only ensue if both blastomeres are viable. Some of these possibilities are schematized in Fig. 14.3.

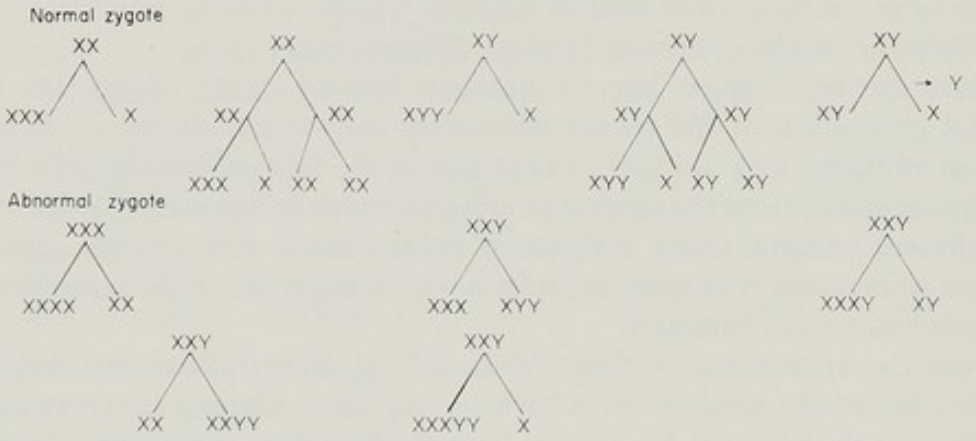


Fig. 14.3. Examples of abnormal mitotic segregation of the X chromosome leading to a mosaic.

The fact that these two accidents, non-disjunction or chromosome losses, do in fact occur is supported by existence of heterokaryotic monozygotism (Chapter 13).

The most probable origin of two monozygotes, one XY the other X0, is that of disturbance in chromosome segregation during the first cleavage of an XY zygote. The XY blastomere would give a normal boy; the blastomere which has become X0 through loss of the lagging Y would be at the origin of Turner's syndrome.

The most probable origin of two monozygotes, one a trisomic 21, the other normal, is that of disturbance in chromosome segregation during formation of the first blastomeres of a normal or triplo 21 zygote. In the first case, one of the first two blastomeres would give the normal twin; the other through disturbance

in segregation a trisomic 21 cell and a lethal haplo 21 cell. In the second case an abnormal triplo 21 zygote would give two blastomeres, one triplo 21 transmitting the anomaly, the other diplo 21 restored to normal through loss of a lagging 21.

The cases of heterocaryotic monozygotism therefore illustrate the third alternative, that of a mosaicism secondary to formation of a normal or pathological zygote. The coupling of these twins gives a mosaic picture in two individuals.

In principle, this third alternative of mosaicism following the formation of the zygote is limited only by the very life of the individual.

Mosaicism between heterocaryotic monozygotic twins may be a consequence of reciprocal blood transfusion followed by a graft (p. 261).

Mosaicism during intra- or extra-uterine life in an individual may be the consequence of any somatic mutation which does not destroy the life of the cell. The mosaic would be more or less extensive and diffuse depending on the general and local conditions of its development.

The responsible mutation may be genomic modifying the actual number of chromosomes: XX/X0 or XY/XXY mosaic. It may be chromosomal producing through translocation a neochromosome, for example, 14 ~ 15/normal. Heterochromy of the irises through irradiation during intra-uterine life is perhaps an example of the latter possibility as reported by Lejeune, Turpin, Mayer and Réthoré (1960f). It may be a genic mutation.

Therefore, to specify the conditions for the appearance and development of chromosome mosaics is to clamour for a solution to frequently insoluble problems.

Before accepting the diagnosis of this mosaicism it is important to eliminate artefacts due to the *in vitro* culture technique (p. 29). Account should be taken only of the proportions of cells higher than those which may be reasonably attributed to chance, which in practice means to verify these proportions in several preparations of the same tissue, and look for the spread of the mosaic in different regions.

However, cytogenetics enables us to explore only certain regions and to cultivate only certain cells with the aid of localized samples.

The development of a mosaic depends primarily on the vitality of its component cells.

One cannot say that certain XXY subjects were not initially a 0Y/XXY mosaic through disturbed segregation at the first cleavage of an XY zygote, since it appears that 0Y cells are not viable. Viability depends also on the medium. This may favour one clone and not another and one cannot infer from what occurs *in vitro* what will occur *in vivo*. This tolerance towards the medium is thought to vary with time. It falls probably with increase in the immunological powers of the individual.

The development of the mosaic depends also on its origin. When it originates very early, simultaneous with the first zygotic mitosis, the mosaicism will not necessarily give an individual half of one chromosome type and half of another. A mosaic type of the morula does not enable us to infer a corresponding type in the individual. The orientation of the plane of the first cleavage of the ovum is not necessarily fixed by the symmetry of its structures. The destiny of some of

the cells of the morula is extra-embryonic, that of the interior cell mass of the blastocyst is in part undetermined.

When it originates later it is probable that differential selection will favour some but eliminate others, and that certain media such as the blood and bone marrow, lend themselves to spread of certain mosaics better than tegument or aponeurosis, and that regional conditions favour certain clones and not others.

It is still impossible to assess the relative value of the many factors capable of bringing about mosaicism and influencing its development.

The new concept of functional mosaicism of the female, active X/inactive X is outlined elsewhere (p. 284).

EFFECTS OF GONOSOMAL ABERRATIONS

Gonosomal aberrations through their varieties and symptoms give information on the value in man of the X and the Y chromosomes.

A. Physiological value of human X and Y chromosomes

1. Sex-determining value

Value of the Y chromosome

(i) The human Y chromosome determines the male sex. An XY zygote, if it is deprived of a Y chromosome, gives an individual of female phenotype and X0 whose ovaries are absent or reduced to a rudimentary stroma.

If deprived of an X chromosome viability is no doubt gravely jeopardized since the 0Y individual has never been observed, although its frequency in the case of disturbance of oogenesis must be equal in principle to that of the X0 aberration.

(ii) Addition to an XY zygote of an additional Y does not give a "supermale". It is usually accompanied by mental debility with hypogonadism.

The masculinizing effect of Y is manifest, even in presence of one or several X chromosomes. The XXY individual no doubt suffers from testicular dysgenesis with sterility, but his phenotype is masculine as is that of XXXY and XXXXY subjects. This fact is amenable to the interpretations considered below.

Value of the X chromosome

(i) An XX zygote if deprived of an X gives the X0 subject mentioned above, in practice dysmorphic and without gonads.

(ii) But addition to the XX zygote of one or even two X chromosomes does not increase the feminization of the phenotype. It disturbs ovarian development and the morphology of the individual less than does loss of an X chromosome. XXX females are often fertile; they differ more from the norm in mental debility than in physical condition.

(iii) No less surprising facts are furnished by male pathology. An X chromosome incapable of changing the masculine phenotype of XXY and even XXXY and XXXXY subjects would have from these examples only a virtually negligible determinant action, sterility apart. And yet its genic value is far from being negligible as evidenced by the number of traits which are linked to it. Thus, more or less hypothetical explanations have been proposed which need to be considered.

2. Interpretations and suggestions

The interpretations proposed deriving from experimental findings are complementary autosomal sex determination, limitation of the activity of the X chromosome by "dosage compensation", and a new concept of "dormant X".

(a) Complementary automosal female sex determination

This notion assumes that the male determinants of the Y chromosome counterbalance both the female determinants of the X chromosomes and of autosomes.

To verify this concept we would need human types with an additional autosomal set, that is, XX3A or XY3A. However, observations on human triploidy which have been reported do not provide the material necessary for study (pp. 288 and 290).

(b) Thesis of "dosage compensation"

To account for the equivalent phenotypic expression of most X-linked genes in XX females and XY males the thesis of "dosage compensation", to coin the expression of H.J. Muller, was proposed. Founded on experimental facts (Stern, 1960a) it implies the existence on the X chromosome of genes capable of cancelling out the effects of different doses of given genes. These genes compensatory for various sex-lined genes are not identical with each other; their locus is thought to be on the X chromosome like that of the genes on which they act. One of the best examples of "dosage compensation" is that found by Muller when he showed that certain variants of the "apricot" eye colour in *Drosophila* depend not on the sex-determing functions of the X chromosome but on modifiers linked to this chromosome.

(c) The concept of the "dormant X"

This concept is based on experimental and human findings. According to its advocates in each cell only one X chromosome is active.

In the female rat one of the two X chromosomes of the somatic cell is said to be inactivated at the seventh day of embryonic life; in the human female at about the twelfth. These estimates are based on the date of appearance of the chromatin body.

This mass of so-called nuclear sex chromatin, which appears at interphase, is said to be formed by condensation of the inactivated heteropycnotic X chromosome.

In the human male and heterogametic males such as the rat, the interphase somatic cell does not contain a sex chromatin mass. This cell, in fact, possesses only one X chromosome of necessity active. This isopycnotic X chromosome does not assume the sex-chromatin mass form at interphase.

Experimental findings

Experimental work supplies cytological and genetic arguments in favour of the thesis of inactivation of the heteropycnotic X chromosome.

(a) Cytological arguments

The chromatin body was discovered by Barr and Bertram in 1949 in the nuclei of the nerve cells of the hypoglossal ganglion. The view that it resulted from adhesion of the heterochromatin portions of the XX chromosome pair was at first held, but soon had to be abandoned.

Ohno *et al.* suggested following extensive studies that this body was nothing other than a heteropycnotic X chromosome from the prophase to metaphase (1959, 1960b, c, 1962c). For their work they mostly used male and female rats, the gonosomal formula for which, respectively XY and XX, is of the same type as in our species.

At the start of mitosis of regenerating liver cells a thick and dark heteropycnotic chromosome can be distinguished in the female rat from the other thin and clustered chromosomes. This is an X chromosome which then becomes isopycnotic when all the chromosomes condense. This chromosome does not exist in the male rat. During meiosis in the female rat the heteropycnotic chromosome does not appear; the two X chromosomes pair off with possibility of recombination. In the male rat, the X and Y are heteropycnotic and do not pair off.

The presence of a heteropycnotic X chromosome was then reported in the cells of the female at metaphase (Sandberg *et al.*, 1960b) or only at the start of prophase (Ohno, 1963a).

These phenomena peculiar to species in which the females are XX and the males XY are thought to be reversed in species with heterogametic females and homogametic males.

(b) Genetic arguments

Parallel with this work the concept of the "dormant X" gradually evolved. This inactivation or in the view of some, this differentiation of one of the two X chromosomes of XX subjects, with its genic consequences of "dosage compensation" was chiefly supported by Mary F.Lyon (1961a, c; 1962). According to this author, only one of the two X chromosomes of the female is active, the other inactivated. This inactivation occurs towards the twelfth day of embryonic life and affects at random the X chromosome of maternal or paternal origin. It is transmitted irrevocably over the cell generations.

This inactive X would correspond to the chromatin body.

The male with only one X chromosome which remains active has no chromatin body.

This thesis at once claimed attention because it helped to interpret certain features of gonosomal aberrations and mosaicism in the female.

It also helps to interpret certain facts noted in the mouse for which the gonosomal formula is of the same type as in our species.

(i) The X0 mouse differs from normal only in having moderately reduced fertility (Welshons *et al.*, 1959). It therefore seems that it can make do with only one active X. The exceptional XXY mouse retains a male phenotype. It is said to be sexually active but sterile (Russell and Chu, 1961b; Cattanach).

(ii) The pigment mosaicism of the coat of the mouse may depend on a certain number of mutant alleles carried at different loci on the X chromosome.

However, the heterozygous mouse is a mosaic of spots of "wild" and "mutant" colour. This suggests that the former are clones derived from cells revealing the activity of the wild allele and the second from cells revealing the activity of the mutant allele. This observation is in good agreement with the thesis of inactivation of one of the two X chromosomes of the female and, in addition, shows that this inactivation apparently affects merely by chance sometimes the X of paternal origin, at others, the X of maternal origin.

In fact, since there are still few cell precursors of the tissues when early inactivation strikes at random one or other X chromosome of the heterozygote, the cell distribution of dominant and recessive characters is unlikely to be equal, reflected in the shapes and colours of the coat of the mosaic mouse.

In the hemizygous males, these mutant alleles are often lethal. In XXY males they have the mottled aspect of the heterozygous female.

Other X-linked traits have led in the mouse to the same observations.

Analysis of the consequences of translocation to the X chromosome of an autosomal gene for coat colour has provided other arguments. In fact, when this X chromosome is heteropycnotic the translocated autosomal fragment undergoes the fate of the X chromosome which carries it (Cattanach; Ohno and Cattanach, 1962b). The result is a mottled aspect for the heterozygous XX female and the XXY male but not for the X0 mouse.

Continuing this analysis, Lyon (1963b) studied the phenotype of the mouse carrying two non-allele mutant genes affecting the same character but each linked to a different X chromosome. The mouse carrying the "tabby" gene on one of the X chromosomes and a gene affecting the structure of the hair on the other is suitable for this control. In line with the theoretical predictions the "tabby" trait is manifest in the coat only at the place where the other gene is not manifest.

In the cat study of a type of pigment mosaicism of the coat (tortoise-shell) gave rise to observations (Frota-Pessoa, 1962) similar to those in the mouse. Heterozygous female cats are tortoise-shell as are the rare heterozygous XXY male cats.

Experimental work, however, provided arguments qualifying the thesis of inactivation. Russell (1963a) studied in the mouse five X autosome

translocations of the type X ~ 1 and one of the type X ~ 8. The results show that the influence of the position of the points of rupture of the X chromosome and autosome was not nullified for any autosomal locus considered. This suggests in his view that functional inactivation does not extend to the X chromosome as a whole. It would spare the segment of the X chromosome which is incapable of inactivating all the translocated autosome. A gradient of genetic inactivation in relation to distance would appear to exist.

Human findings

(a) Cytological arguments

(i) Human cell studies have brought to light facts relating to the heteropycnotic X similar to those discovered in the male rat (Sandberg *et al.*, 1960b; Stewart *et al.*, 1961d; Ohno *et al.*, 1962c, 1963a) (see p. 278).

Just as discovery of the chromatin body in mammals was followed by human applications (Moore *et al.*, 1953) this criterion of the X chromosome led to useful comparisons between its significance and those of the karyotype in gonadosomatic dysgenesis. The chromatin body gives DNA staining reactions. This technique was supplemented by the ^{3}H-thymidine test.

The chromatin body studied in the somatic cell nuclei at interphase in the normale female is plano-convex, it may be bipartite. Its plane face is fixed flush with the inner aspect of the nuclear membrane. Its diameter is about half a micron.

In the best conditions of study it is found in more than 90 cells per 100 (Klinger, 1957). The proportion usually assumed is from 20 to 60 cells per 100. This percentage found in aborted or stillborn female foetuses may fall during the first and second days of life (D. W. Smith *et al.*, 1962a; Taylor, 1963), at the menses (del Campo and Ramirez, 1961), on the eve of delivery and on the 2 days following it (D. W. Smith *et al.*, 1962a); it may also fall under the influence of cortisone and ACTH (Taylor, 1961, 1963). Quantitative and qualitative variations have been noted depending on the age of the cultivated cells (Gautier, 1961b) or under the influence of antibiotics (Sohval, 1961b), and it may be detected in live cells (Schwarzacher, 1963).

The chromatin body does not exist in the somatic cells of the male. Some workers hold that it is not completely absent and put the frequency at 0–4 per cent (Segal, 1957) and at 0·5 rarely exceeding 3 per cent (Klinger and Schwarzacher, 1962).

In the female and also in the male, the need to count not only the peripheral bodies but possible central bodies has been suggested (Fraccaro *et al.*, 1960c). This point of view is still under study.

(ii) The value which the chromatin body may provide as a pointer to the gonosomal formula was verified when it was found that X0 and XYY forms of gonado-somatic dysgenesis were chromatin-negative; the XXY and XXYY forms had one chromatin mass, XXX and XXXY had two and XXXX and XXXXY three. These results showed that the number of bodies in diploid cells

with gonosomal aneuploidies was equal to the number of X chromosomes minus one (Carr *et al.*, 1961d; Jacobs and Strong, 1959f).

This pattern was completed by observations *in vivo* in a heteropolyploid individual (Böök and Santesson, 1960c, 1961e) of a mixture of 3AXXY and 2AXY cells with no chromatin mass and of 2AXXY with one chromatin mass. This fact concurred with the observations *in vitro* of 2AXXYY cells with one (Reitalu, 1957; Muldal and Ockey, 1960) and 4AXXYY cells with no sex-chromatin mass. Thus, in polyploid cells only one active isopycnotic X chromosome is thought to correspond to each additonal set of autosomes (Lennox, 1962).

Other qualitative or quantitative variations in buccal chromatin must be considered.

A percentage below 20 indicates certain mosaicism. An abnormally low or high percentage may reflect certain structural anomalies of the X chromosome. A short or long arm deletion of the X chromosome reduces the size of the chromatin body; a long arm duplication may increase it.

There is concordance between skin gonosomal formula and buccal chromatin, but on the other hand, exceptional discordance between the X0 (skin and blood) formula and positive buccal chromatin or between the X0 formula and positive chromatin of fibroblasts *in vitro*.

(iii) Labelling with ^{3}H-thymidine made it possible to complete in humans the observations which have just been described.

Experimental plant and animal cytogenetics showed that DNA synthesis in various chromosomes or even in different regions of the same chromosome may be asynchronous (Taylor, 1958, 1960; Lima de Faria, 1959, 1961).

Various human cytological investigations have since revealed similar facts (Lima de Faria *et al.*, 1961; Germain, 1962b; Morishima *et al.*, 1962; Gilbert *et al.*, 1962; Atkins *et al.*, 1962d; Moorhead and Deffendi, 1963a).

Using ^{3}H-thymidine and autoradiography it is possible to assess the time of chromosomal DNA replication at interphase and the granule distribution. This method has shown examples of asynchronism between two homologues in all chromosome groups, particularly clear in the somatic cells of the normal female. In these cells, in fact, one chromosome is distinguished by the longest duration of replication. This chromosome found neither in the male nor in X0 subjects is considered as the heteropycnotic X which produces the interphase body. In the normal male, this heteropycnotic X and the Y excepted, the process of "replication" is similar to that in the normal female (Morishima *et al.*, 1962).

Synthesis of DNA of the X chromosome is thought to be achieved at first in the region of the centromere, then completed in the short arm at the same time as in the most distal part of the long arm and then in the proximal part of the latter.

Parallel study of human X aberrations and ^{3}H-thymidine tests has given interesting results. It has been concerned with observation of presumed mosaicisms X0/X**X**^{ii} (Miller *et al.*, 1962c; Muldal *et al.*, 1963; Lindsten *et al.*, 1963d; Giannelli, 1963) and X0/XX/XXX (Morishima *et al.*, 1962). It has also been concerned with numerical aberrations such as XXX and XXXXY (Mukherjee *et al.*, 1963b), XXXXY (Rowley *et al.*, 1963) and XXX (Giannelli, 1963), or structural ones such as presumed X**X**^{ii} (Taft and Brooks, 1963).

These analyses have established that the number of chromosomes of the 6–12 + X group distinguished by a longer replication of DNA is equal to the number of chromatin bodies, that is, the number of X chromosomes minus one.

They also established that the incorporation for each of the arms of a long-armed isochromosome XX^{1i} proceeds in the same way as for the long arm of a normal X.

(iv) Study of the chromatin body is tied up with that of the "drumstick" (Davidson and Smith, 1954). This element is a chromatin appendix attached to the nucleus of neutrophil leucocytes of the female by a thin filament or stalk. Its diameter is about 1·5 μ. In practice, it is absent in the male.

This body is generally considered as equivalent to the X chromosome (McKusik, 1962a). However, its frequency is in no way comparable with that of the Barr body. It varies on average from 0·5 to 10 per cent in the nuclei of the female (Davidson *et al.*, 1961d).

This frequency in the normal state is subject to variations even in the same subject and also between subjects.

In the same female the number of drumsticks increases with the degree of segmentation of the nuclei of the neutrophils (Davidson and Smith, 1954, 1961d; Mittwoch, 1961a). This degree of segmentation of the neutrophil nuclei or "index of segmentation of the neutrophil nuclei" or "index of nuclear segmentation" (NSI) expresses the mean number of lobules per 100 nuclei of polynuclear leucocytes.

The number of drumsticks in a female also varies during the menstrual cycle (Caratzali *et al.*, 1957; Mittwoch, 1961a; Caratzali, 1963); fall at the start of the cycle and rise from the tenth to twentieth day. These fluctuations are not linked with the NSI (Caratzali, 1963).

Differences have also been noted between normal females. They may be of genic origin (Briggs, 1958; Mittwoch, 1961a). After calculating between drumsticks and NSI a correlation coefficient of +0·44 Mittwoch noted that for the same NSI the frequency of drumsticks widely varied between the women considered (Mittwoch, 1963a).

During their description of the drumstick Davidson and Smith also noticed in the female existence of nodules unstalked but sessile. While some workers consider these sessile nodules have the value of incompletely exteriorized drumsticks (Kosenow and Scupin, 1956; Mittwoch, 1963b), others consider that only the drumstick is truly characteristic of the female sex (Davidson, 1961c).

The variations in frequency of this nuclear nodule, stalked (drumstick) or sessile, have been considered in various chromosomal aberrations.

In trisomy 21 its frequency is reduced more than the fall in the NSI would suggest. In fact, for the same NSI the frequency is lower in trisomy 21 than in normal females.

In numerical anomalies of the X chromosome its variations in frequency are less regular than those of the Barr body (Maclean, 1962a). Sometimes, they are even conflicting (Jacobs *et al.*, 1960a; Greenblatt *et al.*, 1962; Carr *et al.*, 1962c; Barr *et al.*, 1962b).

In the X0 subject it is missing.

In the XXY subject the fall in the NSI (p. 193) coincides with a low number of drumsticks (Davidson and Smith, 1959; Mittwoch, 1961a).

The XXX subject according to the results considered in eight subjects does not have an increased number of drumsticks (Johnston *et al.*, 1961c) or is even reduced as shown by examination of eight other subjects (Maclean, 1962a). This fall relates to the drumsticks but may be compensated by increase in relation to normal females in sessile nodules (Mittwoch, 1963b).

The possibility of a double drumstick has been reported (Jacobs *et al.*, 1959c), then questioned (Davidson, 1961c), then confirmed (Maclean, 1962a). Not only the double drumstick but also large drumsticks have been found (Mittwoch, 1963b).

Study of three XXXY subjects led Mittwoch to results similar to those of her study of the XXX subjects. This inability to form the filament of the drumstick is said to go hand in hand with fall in nuclear segmentation. But wherever segmentation is limited, formation of drumsticks is more inhibited than nuclear segmentation. Furthermore, in females with Pelger's anomaly, the frequency of drumsticks is much lower than the level which would results from predominance of bilobate cells: zero frequency (Davidson and Smith, 1954) or 0·15 per 100 (Lüers and Petzel, 1958).

The variations in the drumstick in relation to anomalies in structure of the X chromosome concur better with those of the chromatin body. They have led to the hypothesis of an X chromosome formed from a heterochromatic long arm and a short euchromatic short arm (Maclean, 1962a). This hypothesis has been refuted (Ohno, 1962a) but the arguments advanced in a subsequent note (Miles, 1962b) show that this discussion is not closed.

(b) Genetic arguments

Observations in support of the "dormant X" thesis have also been made in the human species.

(i) Some are provided by study of glucose-6-phosphate dehydrogenase (G-6-PD). Production of this enzyme depends on a gene the locus of which is situated on the X chromosome. When this normal gene is replaced by an abnormal allele, the level of G-6-PD in the red cells of females homozygous and males hemizygous for this pathological allele is nil. Their red cells can no longer properly reduce glutathione. These subjects are then sensitive to primaquin.

The thesis of inactivation of an X, indifferently paternal or maternal in the female, accounts for facts which had so far received no satisfactory explanation. These facts are outlined in the following chapter (Chapter 15).

(ii) This hypothesis of a mosaic effect in the female heterozygous for an X-linked trait is compatible with other observations (Lyon, 1962).

One of the most demonstrative is that of sex-linked albinism of the eyes. The retinal epithelium of the affected male is devoid of pigment; that of the heterozygous female is irregularly pigmented, the mixture of spots with and without pigment giving a dotted appearance (François and Deweer, 1953; Waardenburg and Van den Bosch, 1956).

Equally suggestive results are provided by clinical and chemical study of

perspiration of females heterozygous for the character "anhydrotic ectodermal dysplasia" (Motulsky *et al.*, 1962b).

Study of serum enzymes in relatives of subjects with pseudo-hypertrophic myopathy of the Duchenne type is said to have disclosed heterozygotes in a large number of cases (Demos *et al.*, 1962).

Finally, assay of G-6-PD in 16 subjects with an abnormal number of X chromosomes showed that all except one had a normal enzyme value (Grumbach *et al.*, 1962a). This study, the last result apart, agrees with the thesis of enzymatic activity of only one X chromosome, the other heteropycnotic and inactivated X chromosomes each giving a chromatin body.

(iii) The discovery in 1962 by Mann and Race and their teams of an X-linked erythrocytic antigen provided cytogenetics with a new "marker", dominant character. It revealed in the female facts which link up with those which Russell (see p. 280) noted in the mouse. The females heterozygous for the Xg group do not have two erythrocyte populations, one Xg(a+) and the other Xg(a−). All their red cells are Xg(a+). This suggests that random inactivation of one of the X chromosomes is not complete and that both genes remain active in certain loci. Thus, the hypothesis of the "dormant X" would appear to be valid only for certain regions. It needs to be verified at the level of the locus rather than at cell level (Reed *et al.*, 1963).

(iv) Subject to the findings of work now in progress, these facts lead to the concept of a functional X/X mosaic female. One of the two cell populations would have for the active X the paternal X and the other the maternal X. But even assuming that the process of inactivation depends only on chance we may suppose that the concordance of X/X mosaicism between two monozygotic twins is not absolute. Inactivation in effect would appear to occur very early. It would intervene when the precursors of the tissues are still few in number. It would be irrevocable and transmitted to all the descendants of the cells.

Slight differences for X-linked characters must therefore be more conspicuous between monozygotic twin sisters than monozygotic twin brothers. Search along these lines may provide many arguments in support of the thesis of Lyon (Vandenberg *et al.*, 1962).

(d) Notes

(i) The thesis of inactivation of an X chromosome at once excited interest. Based on examples of mosaicism noted in the female and also in the mouse heterozygous for the X-linked mutant alleles, it had the great distinction of explaining the variations in chromatin body depending on the types of chromosomal aberration. In diploid cells there are as many chromatin bodies as X chromosomes minus one, in triploid cells there are as many chromatin bodies as X chromosomes minus two.

(ii) After this hypothesis was accepted a first objection immediately arose. If the diploid cell requires only one active X why the differences between X0, XX and XXX; between XY, XXY, etc? This objection was at first met by the hypothesis of a poor start to embryogenesis. Before the chromatin body appears,

i.e. during about the first 12 days of its life, the human XX embryo would require two active X and the XY only one. Any error in distribution of X either in the sense of more or less chromosomes would then put the development along a pathological road to Turner's syndrome, Klinefelter's syndrome, etc., while the X0 mouse would remain fertile because 7 days would be less harmful to it than the 12 days for the human X0.

(iii) The thesis of total inactivation was then replaced by partial inactivation in the light of new experimental and human knowledge. In this case, the X0 would suffer during its life from absence of a small segment of X, the XXY from presence of two of these segments, the XXX from a partial trisomy of X. But some workers then raised the objection that X0 and XY, XXY and XX, etc., have the same total X activity and that whereas some have disturbances in development, others do not have any at all.

(iv) An additional hypothesis was then put forward, that of the presence on the Y of a segment homologous with the active segment of the X chromosome. This thesis ironed out certain difficulties but not all; cytogenetics provides no evidence of a paired segment for X and Y apart from the extremity of these chromosomes (end-to-end pairing).

(v) Another explanation was then proposed (Gartler and Sparkes, 1963). During the inactivation the single X of the X0 subject would be active or non-active according to chance. Only cells with an active X would survive. The embryo thus deprived of about half its cell capital may survive stunted and malformed, but would now be made up only of cells possessing an active X; XXX and XXXX individuals would not undergo the same early damage but would, on the contrary, retain their abnormal cells indefinitely. This difference makes plain the phenotypic and mental dissimilarity between X0 subjects, on the one hand, and XXX and XXXX subjects, on the other.

The cellular features of $X\mathbf{X}^{i}$ subjects have been explained along similar lines (p. 220). But this explanation which extends inactivation by chance to the single X of the X0, does not tell us why the XY itself escapes this nullifying process which should deprive it at the start of embryogenesis of half its cells. It assumes that the principle of inactivation by chance, essential to Lyon's thesis, does not apply to the long-armed X isochromosome ($\mathbf{X}^{i}$). These remarks were developed by Rohde and Berman (1963b) who conclude that inactivation must spare the X0 and XY cells, be selective for certain X chromosomes of abnormal structure and proceed by chance when at least two X of normal structure are present.

The problems of the chromatin body (Muldal, 1962a) and drumstick (Mittwoch, 1963b) and of the "lyonization" of the Y chromosome (Rashad, 1963; Burch and Burwell, 1963b; Gray, 1963; Lyon, 1963a) are still under study.

Lyon's thesis not only had the advantage of proposing an attractive explanation for certain effects of X aberrations but of setting in train a whole number of ideas.

3. Effects of the gonosomal type on sex development

At the start of embryogenesis the chromosome sex determines the masculine or feminine orientation of development of the "bipotential" rudiments of the gonads and their adnexa.

This determination is indirect. It is achieved through inducer substances, hormones, which transmit the chromosomal information to the "receptors", the competent tissue.

Wolff and Ginglinger continuing the work of Ancel and Bouin showed in a series of studies initiated in 1935 in birds that it was possible by injecting crystalline female hormones to feminize an embryo of masculine chromosomal sex. The genital organs of the male embryo thus treated are at hatching a typically female ovary and oviduct. But during development they gradually take on the masculine form.

Conversely, it is possible by injecting crystalline male hormone to masculinize an embryo of female chromosomal sex.

This would suggest that the chromosome sex difference is expressed in a chemical difference before it is reflected in a morphological difference.

Subsequent experiments confirmed this hypothesis. They showed that the masculine or feminine development of the gonads and adnexa is achieved via hormones, androgens or oestrogens, depending on the gonosomal constitution of the embryo and also that these chemical intermediaries are formed by the gonadal rudiments.

On the one hand, grafts of embryonic testicle or ovary produced the same inversion as injections of male or female crystalline hormones. The destruction of the primary gonads, on the other hand, leaves intact after development of the adnexa only the non-hormonal part common to both sexes.

On the other hand, gonads cultivated *in vitro* become differentiated into ovary or testicle depending on their chromosome type. They therefore harbour the factors essential for their differentiation. These hormonal substances, androgens or gynogens, are thought to be elaborated by the medullary somatic cells (Haffen, 1962). This natural evolution may be reversed by addition to the culture medium of natural or synthetic hormones and also by parallel culture in the same medium of undifferentiated female and male gonads. The female gonad assumes the ovarian type while the male gonad becomes an ovotestis or even an ovary.

These facts have been verified on many lower vertebrates and in a less complete manner, marsupials apart, in higher vertebrates. In the latter, additional findings have been obtained. They are of interest since they clarify to some extent the pathogenesis of human gonado-somatic dysgenesis.

According to the experiments of Jost (1947, 1958) very early bilateral castration of the male or female rabbit embryo before a stage corresponding to that of the human embryo of 15–30 mm is followed, whatever the chromosome sex, by the formation of a female phenotype. Müllerian development does not require gonadal hormonal intervention. Wolffian development, on the other

hand, would require at a later stage secretion by the foetal testicle of two varieties of inducer substances.

One would produce Wolffian stimulation. It would appear to be similar to testosterone since its action may be reproduced in the castrated animal, whatever its sex, by implantation of testosterone; the other of Müllerian inhibition, the effects of which can be produced in the castrated animal only by implantation of a testicular graft.

The development of the gonads, their adnexa and then secondary sex characters continues both in space and time. It is subject to fundamental space-time demands.

(a) The effects of castration or graft are limited to the castrated or grafted side. Unilateral extirpation of the foetal testicle is followed by Müllerian development on this side. The testicular graft on the side of on ovary exerts an action on the Wolffian structures of this side only. The active principles of the foetal testicle act on the Wolffian system by a local, unilateral and descending route. Their efficacy decreases from top to bottom. The proximal Wolffian derivatives develop more easily than the distal ones. On the other hand, these principles act on the urogenital sinus by a general route.

(b) Time also directs the different acts of this process.

1. Secretion of the foetal testicle is asynchronous. The principle of Müllerian inhibition precedes that of Wolffian stimulation.

The first, if we go by experimental work (Jost, 1947, 1958, 1953; Raynaud and Frilley, 1947), is secreted from the 50th–60th day of foetal life, the second from the 60th day.

2. The differentiation of the receptors is also asynchronous. But the responses of the epididymis, the vas deferens, seminal vesicles, prostate and external genitals to the inducer substances require rigorous concordance which the slightest mistiming may seriously upset.

Differentiation of uterus and tubes precedes that of the urogenital sinus. From the start to the end of growth this resonance between inducers and receptors must operate. However, the risks of disharmony of growth become less as the pace of development slows down.

Certain additional ideas must be recalled since they may explain apparently paradoxical facts. Androsterone is capable in the bird of an ambosexual action: masculinizing in females, feminizing in males. Its masculinizing action is mainly exerted on the genital tract of the female and it produces more or less marked atrophy of the Müllerian canals. Its feminizing action is mainly exerted on the gonads of the males causing transformation of the testicle into an ovotestis.

The threshold of the masculinizing action is lower than that of the feminizing action and the latter varies according to the race treated.

Another problem, that of the possible role of differences between chemical structure of an embryonic hormone and the structure of the adult homologous hormone has not been elucidated. Some (Wolff, 1962) defend the idea of hormone identity. The examples of pseudo-hermaphroditism of newborn, in particular, in females, through androgenic treatment of the mother during pregnancy, do not contradict this thesis. The consequences of this treatment are

similar to those of injection of its male hormones by the foetal calf to its twin sister via the interplacental vascular connections. This twin will be a "free-martin", a sterile animal with atrophied ovaries, peniform clitoris and male phenotypic orientation. It was recently possible to reproduce in the laboratory (Salzgeber, 1962) what nature does in producing free-martins, an example of feminine pseudo-hermaphroditism.

According to these experimental facts it is conceivable that any quantitative or qualitative anomaly of the chemical intermediary, inducer substance with the task of transmitting gonosomal information, may affect development and sex orientation of the gonadal rudiments and their adnexa. It is also conceivable that other receptors, such as the encephalic ones, may themselves also suffer at a very early stage from the gonosomal aberration.

4. Comparative value of the X and Y chromosomes of human and certain experimental species

Certain experimental notions may be recalled insofar as they help us to understand certain human facts.

(a) The female *Drosophila* is XX homogametic and the male fly XY heterogametic. The feminine determinants are carried by the X, the masculine by the autosomes; the Y does not carry any but transforms the sterile male X2A fly into a fertile fly (Morgan *et al.*, 1925).

The male determinant action of the autosomes was demonstrated thanks to the phenomenon of triploidy. An additional autosomal set A may tip the balance in favour of masculinity. It transforms the normal XX2A female into an intersex XX3A fruit fly.

The sex phenotype of *Drosophila* depends therefore on the ratio—number of X chromosomes/number of autosomal sets. If this ratio is equal to 1, the fly is female: XX2A is the normal female; XXX3A is the triploid female. If this ratio is equal to 0·5 the fly is male: X(Y)2A normal male; X2A sterile male; XX4A fly of male phenotype.

The variations in these ratios have phenotypic and functional consequences: the 3X2A fly known as "superfemale" is hypogonadal, sterile and malformed; the 2X3A or 3X4A fly intersexual; the X3A fly is known as "supermale" but it is also sterile and malformed. These factors of sex determination may be modified by mutations; the genetic female fly which possesses in the homozygous state the "transformer" gene (tra) (Sturtevant, 1945) has a male phenotype. This gene has no effect on genetic males.

(b) The female plant *Melandrium* is homogametic XX and the male plant heterogametic XY; the female determinants are carried by the X, the masculine by the Y. The influence of the autosomes on the balance between them is secondary: whether there are 2A, 3Aor 4A, the XX is always female, the XY male. In this species the balance is mainly ensured by the X and the Y. A slight autosomal masculinizing effect appears in the XXY2A, 3A or 4A individuals and in XXXY3A or 4A individuals which give male plants with some intersex

flowers and in XXXXY4A individuals which give intersex plants with some male flowers.

(c) The gonosomal formula of the mouse is of the human type; the female is homogametic XX, the male mouse heterogametic XY; the phenotype of the X0 mouse is feminine and compatible with reproduction, that of the exceptional XXY masculine but apparently accompanied by sterility (p. 193).

Demonstration of autosomal sex determinants in this rodent runs into experimental difficulties. Triploidies are no doubt not rare but they succumb before the gonads are sexually defined and before it is possible to compare the sex phenotypes of the combinations 3A with the gonosomal sets XXX or XXY, etc.

Mammals as a whole appear to be modelled on the human, but X0 males have been observed (Matthey, 1949).

These experimental notions bring out the general character of this necessary equilibrium of feminizing to masculinizing factors, they draw attention to the possible role of autosomal sex determinants and to the role of mutants capable of reversing the sex phenotype. Finally, it would not be complete if we did not recall that these determinants are not insensitive to the environment. Temperature influences the phenotype of *Drosophila* 2X3A; if it falls it favours masculine orientation, if it rises, the feminine orientation of this phenotype.

B. Pathological effects of numerical anomalies of X and Y chromosomes

The cytogenetic evidence so far gathered suggests new ideas rather than offering precise information. Some of these findings are based on a very small number of observations or even only one. Others need to be verified or are only the initial results of studies in progress. Others raise problems which will be solved only when techniques remove the risk of misinterpreting mosaics or aberrations which elude present analysis. Another difficulty is delineating the symptoms noted.

Some are common to several types of aberration. They suggest that different genic imbalances may have similar dysembryogenic consequences.

Others are directly attributable to the imbalance of feminizing to masculinizing factors. They alone will be considered.

With these reservations it is possible to discuss the relations of gonosomal numerical anomalies with viability, with gonadal-somatic dysgenesis and with hermaphroditism.

(a) Viability

By and large the human species appears to cope better with numerical anomalies of the gonosomes than of the autosomes.

The imbalances produced by the absence of a Y in the male and an X in the female or in both sexes by one, two or three X chromosomes in excess are compatible with life and development.

The mouse, which is the best experimental model of human gonosomal anomalies, also shows similar tolerance: viability of the XXY and X0 types and fertility, even though a little reduced, of the X0 type.

This relative tolerance and these possibilities of adaptation, which suggest interpretations previously outlined (p. 279), do not, however, exclude lethality. A good number of varieties started off by processes of non-disjunction or chromosome loss have never been found (pp. 272, 273 and 274). It is possible that this difference between the number of known types and possible types will be reduced by future discoveries. It is also possible that it may never be completely bridged since certain varieties are lethal. This is most probably true of the 0Y, the frequency of which is theoretically equal to that of X0, an assumed disturbance in oogenesis being involved, and which has never been found.

(b) Gonado-somatic dysgenesis

On the preceding experimental bases it is possible to attempt interpretation of the consequences of X0 and XXY and allied anomalies.

These anomalies through abnormal genic dosages which they produce inevitably upset development and cell function. Certain regions are more particularly affected because of their sensitivity, their phase of development, or both.

The primary gonads figure among these sensitive organs. Various hybrid species are sterile because of genic imbalance which impairs their gonadic without disturbing their somatic development.

X0 and related types

In the light of experimental evidence, the chromosome aberration X0 through an insufficiency or alteration of inducer substances impedes differentiation of the gonadal rudiments at a very early stage. Development of the cortex and the medullary regression do not occur. The development of other receptors associated with these inducer substances is in turn disturbed. It will mainly continue, to use the expression of embryologists, at the level of the non-hormonal regions of the Müllerian adnexa, those which are common to both sexes.

With time the gonado-somatic dysgenesis will be completed under the influence, in particular, of severe hormonal disturbances triggered by ovarian insufficiency.

Ovarian damage of the X0 type is, in general, profound if not complete. However, exceptions have been reported with, in place of "fibrous trails", ovarian histological structures and even exceptionally functional ovaries (Bahner *et al.*, 1960b; Hoffenberg *et al.*, 1959; Stewart, 1960d).

To explain the exceptional examples of functional ovaries, the role of hypothetical autosomal feminine determinants has been suggested. If we have human types with an additional autosomal set, XX3A and XY3A subjects, they would enable us to assess the value of this hypothesis but the present information on human triploidy does not provide this possibility. However, in principle, we

cannot dismiss the possibility of autosomal feminine determinants or consider without reservation X0 subjects as neuter subjects.

But another hypothesis more in line with the findings and the present possibilities of cytogenetics also accounts for these Turner syndromes with functional ovaries: that of an unrecognized XX/X0 mosaic.

Gonads and their adnexa apart, the brain is amongst the organs most sensitive to numerical X anomalies, not so much through absence as through excess. It is, in fact, worth noting the frequency of mental deficiency among XXX and XXXX subjects.

Originally, XXX subjects were described as "superfemales" but this term has been criticized (Stern, 1959b).

3X2A *Drosophila* despite feminine to masculine determinant ratio higher than that of the normal female 2X2A fruit fly, does not merit the name "superfemale" given to it. These flies are in fact hypogonadic and sterile with abnormal chaetae, wings and eyes.

The phenotype of XXX and XXXX females is also a reflection more of damage produced by an aneuploidy, than of excess of feminizing factors over masculinizing ones.

The normal XX and XY feminine and masculine types have reached the sex differentiation boundary which a higher dose of genetic determinants of sex cannot cross.

Endocrinologists have accommodated next to the X0 in the category of Turner's syndrome an XX variety. It is premature in the present state of our knowledge to estimate the nosological value of this variety.

Certain familial observations, for example, that of two sisters both chromatin positive, have been considered as perhaps deriving from a genic cause. Another observation relating to the discovery of the syndrome in two sisters, one chromatin-negative, the other chromatin-positive (Schönenberg), is more suggestive of coincidence in this sibship of an X0 type and an XXX type.

A case of XX "male Turner's syndrome" has been considered (Oikawa and Blizzard, 1961) as the possible human replica of the XX *Drosophila*, homozyous for the gene (transformer) which causes inversion of the feminine to the male phenotype. The possibility of a Y ~ X translocation with transfer to the X of the male determinants could also be considered, or the possibility of a concealed mosaic. However, a further check revealed a mosaicism involving a clone with abnormal Y chromosome (Solomon *et al.*, 1963).

Subjects with Turner's syndrome of normal XX or XY constitution have been explained by sufficiently early lesion of the primary gonads, embryopathic equivalence of the experimental destruction of the gonadal rudiments. Observations of XX or XY "pure gonadal dysgenesis" appear to stem from this aetiology. These various suppositions are only working hypotheses.

XXY type and related types

The XXY gonosomal aberration through insufficiency or hypothetical change in inducer substances, may impede testicular differentiation of the medulla and processes of Wolffian stimulation and Müllerian inhibition.

These initial dysgeneses are thought to be supplemented as growth continues by abnormal development of other receptors and hormonal imbalance induced by the primary gonadal anomaly. The XXY aberration creates a gonado-somatic dysgenesis rather than a type of intersex (Stern, 1961).

The XXY subject despite his small testicles, atrophy of the seminiferous tubules and sterility, is still a male. He is sexually active as is the XXY mouse. He may assume a eunuchoid type but his gynaecomastia is an ordinary consequence of testicular hypoplasia and not a sign of primary feminine determination. Fertile XXY subjects (?) have even been reported, they are discussed elsewhere (p. 193).

The XYY subject according to rare descriptions, does not differ from normal or is a cryptorchid mental defective. He therefore does not merit the description "supermale" any more than does the X3A hypogonadic sterile and malformed *Drosophila*.

Klinefelter's syndrome, along with the best defined XXY type and the allied types XXXY and XXXXY, involves varieties without gonosomal numerical anomalies. They are so little known that they warrant only surmises: that of an X-linked form transmitted by females of which a familial observation provides some examples (E. G. Reifenstein, Jr., 1947); that of early acquired forms through gonadal embryopathy or late form through orchitis, a possibility suggested by the clinical and hormonal consequences of bilateral orchitis due to mumps in adults (Decourt, 1960a); that of acquired forms through cerebral cortical or hypothalamic lesion in subjects with normal genital development. The testicular atrophy which develops is based on that of a Klinefelter's syndrome. The hypophyseal gonadotrophic function is intact. The aetiological process appears to be strictly neuro-germinal without hormone intervention (Sendrail, 1962a).

(c) Hermaphroditism

The hermaphrodite has the normal equipment of both sexes, anatomical and functional. He is capable of producing at one and the same time simultaneously male and female gametes like monoic plant species.

Hermaphroditism is the rule in certain zoological groups, especially in fixed animals or parasites (plathelminth annelids, gastropod molluscs, etc.). In other groups it is observed only in a few species (sponges, isopod crustaceans, lamellibranchi). It is rare but not unknown in amphibians, birds and fishes.

Hermaphroditism in the strict sense of the term does not exist in the human species. The human embryo following a fundamental condition of the metazoa possesses at the start of development ambivalent sexual potentialities. The sex which will be overt will be that which will dominate over the other. This will be that of the gonosomal formula.

In certain pathological conditions dominance of one sex over the other does not come about. The individual is equipped both with male and female gonads, more or less abnormal. These gonads are not functional. Humans are therefore not hermaphrodite but intersex.

To conform with usage we shall retain the term hermaphroditism which some even call "true hermaphroditism".

Cytogenetic studies lead one to distinguish hermaphrodites with and without chromosome mosaics (Chapter 12).

(a) Hermaphrodites with mosaics consisting of two clones, one with one or more X but without Y, the other with one or more X and one Y, do not upset established notions. In man, in fact, as also in the mouse, the Y appears to be essential for testicular development.

The X0/XY and XX/XXY mosaics, probable consequences of a mitotic anomaly at the first cleavages of a normal or abnormal zygote, are thought to control gonadal ambosexuality followed by anatomo-clinical ambosexuality.

A comparison may be made between the X0/XY hermaphroditism and a pair of twins "heterokaryotic monozygotes" (Turpin *et al.*, 1961e, 1963b) consisting of a normal XY boy and an X0 Turner's syndrome (Chapter 13).

XX/XY hermaphroditism is amenable to the interpretation suggested by the coincidence of the gonosomal XX/XY mosaicism and mosaicism of erythrocyte and serum phenotypes (Chapter 12), leading one to consider that this XX/XY may be the result of double fertilization prior to elimination of the second polar body or fusion into a single embryo of two zygotes of opposite sex. This joint development would be the human equivalent of the picture seen *in vitro* (Wolff, 1962) by simultaneous culture of two still undifferentiated gonads, the one of feminine gonosomal formula, the other of masculine formula. The first normally differentiates into an ovary and more or less affects the testicular development of the second.

Mosaicism according to the observations reported therefore provides an attractive interpretation of hermaphroditism. It does not contradict the control of the masculine orientation of the primary gonad by the Y chromosome.

But a possible objection to this view is that such mosaicisms have been reported in subjects considered to have gonado-somatic dysgenesis often evocative of Klinefelter's or Turner's syndrome.

In fact, many of these observations do not mention the results of anatomical examination of the gonads, or only give results often incomplete of a unilateral examination.

Even with the aid of multiple samples present-day cytogenetics is incapable of evaluating the diffusion at all points over the organism of each of the clones isolated. It is possible that ambosexuality appears only if the balance of masculinizing to feminizing factors comes between certain limits and in certain regions (gonads).

(b) The interpretation of hermaphroditism without mosaic is even more difficult. It has been seen a little more often than the preceding type. In addition, these are chromatin positive subjects with XX formula.

The first idea which comes to mind is that of apparent absence of the Y chromosome.

Some of these subjects will be in reality mosaics but the clone containing the Y may exist only in unexplored regions. It must be recognized that many of these observations are based on exploration of only one region, most often the

bone marrow and very rarely, gonadal tissue. Various authors do not discount this possibility: XX/XY or XX/XXY mosaics, for example, masked by a semblance of an XX uniform type.

Others may be the consequence of reciprocal translocation between X and Y, which would supply the X with masculinizing factors and consequently give intersex XX2A genotypes.

Such accidents lead us to consider the oft championed hypothesis of the genotypic coincidence of feminizing and masculinizing factors both in the male and female. According to this hypothesis any genotype is intersex and sex depends on the predominance of one system over the other. Experimental facts are said to indicate (Seiler, 1958; Hannah-Alava and Stern, 1957) that intersex at cell level may be expressed in strict male or female differentiation.

In the eventuality of a balance between the two systems, fortuitous non-genic local factors, even minimal, at the start of embryogenesis may tip the balance in one direction for one gonad and in the opposite for the other. This hypothesis would explain the ovotestis. It is even said to explain the possibility of XX subjects with two testicles (Shah *et al.*, 1961).

Other observations, that, for example, of coincidence of hermaphroditism in two or even three sibs, appear to be the replica of the genic hermaphroditism observed in the animal, the pig, in particular (Chapter 12).

The causes of pseudo-hermaphroditisms, in particular, the genic nature of some of them, have been considered together with the other characters which distinguish them from hermaphroditism.

CHAPTER 15

Chromosome-linked Biochemical Effects

BIOCHEMICAL genetics, as a rule, deals with transmission of mutant alleles leading to qualitative modification of an enzyme the synthesis of which they direct. In the case of an unfavourable allele this modification is reflected in partial loss in the heterozygote or total loss in the homozygote of enzymatic activity.

Chromosome anomalies bring into play the entire normal or abnormal genic content of the chromosomes or parts of the type involved. In numerical anomalies, trisomies and polysomies, surplus amounts of genes are found which may be expressed in over-production of various enzymes. On the other hand, structural anomalies by translocation or deletion may be accompanied by loss of part of the genic material and reduced enzyme production. This quantitative relationship between enzyme synthesis and hereditary material contained in the nuclear figured elements—the chromosomes—presupposes that the various chromosomes or part of a chromosome are equally active. It would appear that such is not the case for the sex chromosomes and that the hereditary substance may be inactivated at least partially in the interphase.

BIOCHEMICAL EFFECTS OF AUTOSOMAL ANOMALIES

The biochemical effects observed to date all correspond to numerical or structural anomalies in chromosome 21. These results do not appear to indicate that chromosome 21 plays a particularly important role but rather that its small size may undergo through deletion or trisomy modifications compatible with the development of organisms permitting their study.

A. Chromosome 21 and alkaline phosphatase

Multi-nucleated white blood cells display high alkaline phosphatase activity which can be measured by histochemical methods (Hayhoe and Quaglino, 1958) or by quantitative biochemical assay on a suspension of washed white cells (Haight and Rossiter, 1950; Valentine and Beck, 1951). In chronic myeloid leukaemia this enzyme activity is very weak (Valentine and Beck, 1951). Recently, chromosome analysis of blood and bone marrow cells has demonstrated in this type of leukaemia existence of clones affected by deletion of the distal part of the small acrocentric chromosome 21 (Baikie *et al.*, 1960; Nowell and Hungerford, 1960). In the light of these facts and the presence of a trisomy involving

this same chromosome 21, one might suspect an increased activity of leukocyte alkaline phosphatase in patients with this trisomy. The results of several authors have validated this hypothesis (Table 15.1).

TABLE 15.1

Alkaline phosphatase activity of the polynuclears of trisomic 21 subjects compared with controls

Authors	Trisomics 21		Controls		Method
	Female	Male	Female	Male	
Alter *et al.* (1962)	64**		24		Biochemical •
Trubowitz *et al.* (1962)	60 ± 27** 70 ± 38**		40 ± 17 40 ± 15		Biochemical •
Lennox *et al.* (1962)	69 ± 16***		48 ± 12		Histochemical, results in arbitrary units
King *et al.* (1962)	112 ± 41*	120 ± 41**	92 ± 36	91 ± 42	Histochemical, results in arbitrary units

• mg of P released per hour and by 10^{10} polynuclear neutrophils.
* $P < 0{\cdot}05$, ** $P < 0{\cdot}01$, *** $P < 0{\cdot}001$.

Without considering experimental differences the ratio found between the overall results in trisomy 21 and controls is 1·65 and close to the ratio of genic material for chromosome 21 which is $\frac{3}{2} = 1{\cdot}5$. It is tempting to consider that the gene or genes directly or indirectly controlling synthesis of leukocyte alkaline phosphatase are located on chromosome 21. Reduced enzymatic activity observed in myeloid leukaemia where there is absence of the distal fragment of chromosome 21 suggests that the position of this gene is well removed from the centromere. Various factors may influence leukocyte alkaline phosphatase activity. ACTH and the corticosteroids lead to hyperactivity. Such activity is also seen during pregnancy, probably due to the effect of progesterone. However, the choice of subjects studied excluded superimposed hormone effects and the results indicate no sex differences. Alter *et al.* (1963) noted that there was a negative correlation before puberty between age and alkaline phosphatase activity which might be dependent on sex hormones but it would appear that the groups studied in the various tests were all of comparable age.

Infections bring a heavy rise in specific enzyme activity in the polynuclears. A particular sensitivity to infections might explain the high levels found in trisomy 21. However, the children were chosen free of all infection and no correlation was found between alkaline phosphatase activity and the polynuclear count.

Maturation of the leukocytes may be involved since Arneth's formula deviates to the left in trisomy 21 (Turpin and Bernyer, 1947) but there is no correlation between enzymatic activity and the index of nuclear segmentation.

More puzzling are the changes in enzymatic activity found in chronic myeloid leukaemia where remissions are accompanied by return to normal in alkaline

phosphatase levels while high levels of activity are seen during the acute transformation phase. In remissions due to therapy it is likely that the raised enzyme activity is connected with changes in the cellular environment independently of the variation of the number of cells carrying a Philadelphia deletion of chromosome 21. In acute flare-ups there may exist an additional important chromosome rearrangement. It is possible that the chromosomal rearrangement involves a direct genic command of the synthesis of alkaline phosphatase while chromosome 21 exercizes indirect control.

Although in chronic myeloid leukaemia only the cells of a pathological clone carry a deletion in chromosome 21, the fall in enzyme activity is very marked. There is no proportionality between enzyme activity and amount of genic material which must be at least equal to 1 in leukaemia, 2 in the normal subject and 3 in trisomy 21. According to the hypothesis of Beutler (1963) there may be random inactivation of one of the 21 chromosomes at an early stage of embryonic development. The deletion if it affects an active chromosome 21 would give a clone not under growth control while it would pass unnoticed if it damaged an inactive chromosome. It is difficult to use this conception to explain the results observed in trisomy 21. It is probably more reasonable to suppose that genes located on the chromosome control other genes which govern the synthesis of alkaline phosphatase. This control would itself be under the influence of general regulatory actions such as hormonal effects.

Clonal somatic anomalies of the karyotype may be of particular importance in establishing relations between the production of cell enzymes and the significance of chromosomal rearrangements.In fact, anomalies incompatible with embryonic development may permit survival of the cells. *In vivo*, such anomalies can be observed in malignant tumorous proliferations and their analysis could bring new facts into the study of cancer. *In vitro*, it is possible to obtain by long-term culture heteroploid strains with a variable number of chromosomes of diverse types and with very different enzymatic activities. Under these conditions the link between the karyotype and cell phenotype is independent of the effects of physiological regulation of the whole organism.

TABLE 15.2

Alkaline phosphatase activity of the cell lines EUE, Sub. 1 and Sub. 2

Cell line	Limits of alkaline phosphatase activity
EUE	75·0 to 120·0
Sub. 1	0·4 to 0·7
Sub. 2	0·1 to 0·5

An *in vitro* study has been undertaken along these lines on the alkaline phosphatase activity of the cells (de Carli, Maio and Nuzzo, 1963). While a primary culture of human fibroblasts possesses only low alkaline phosphatase activity,

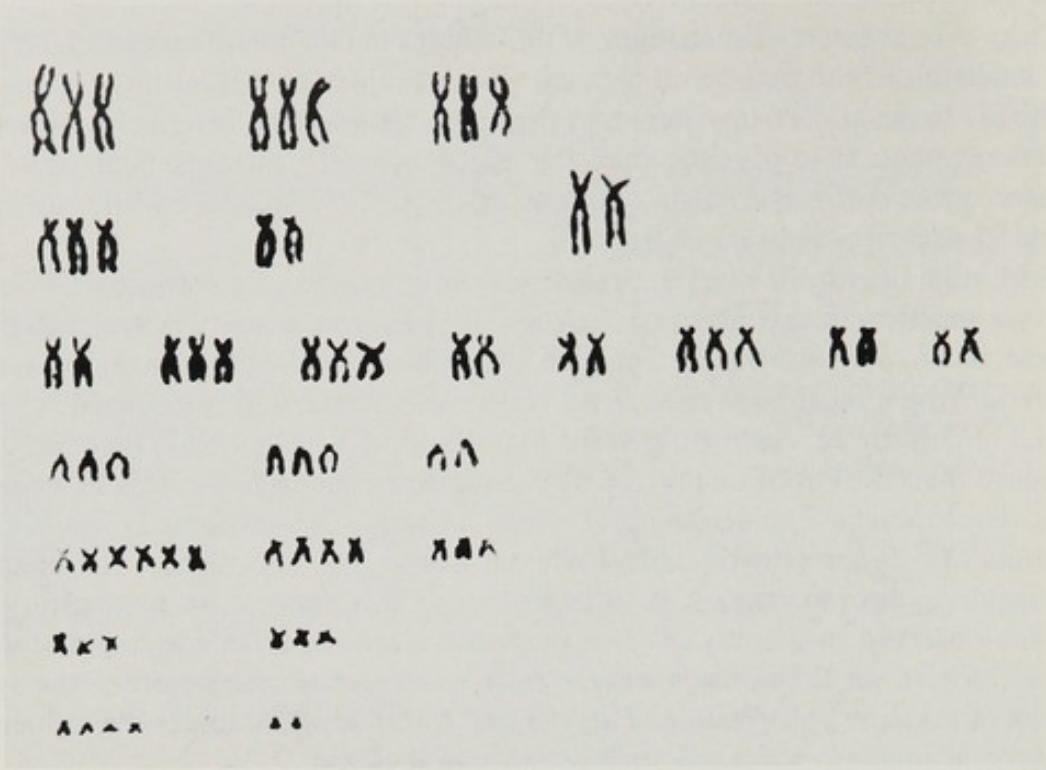


FIG. 15.1. Karyotype of the EUE heteroploid strain (de Carli *et al.*, 1963).

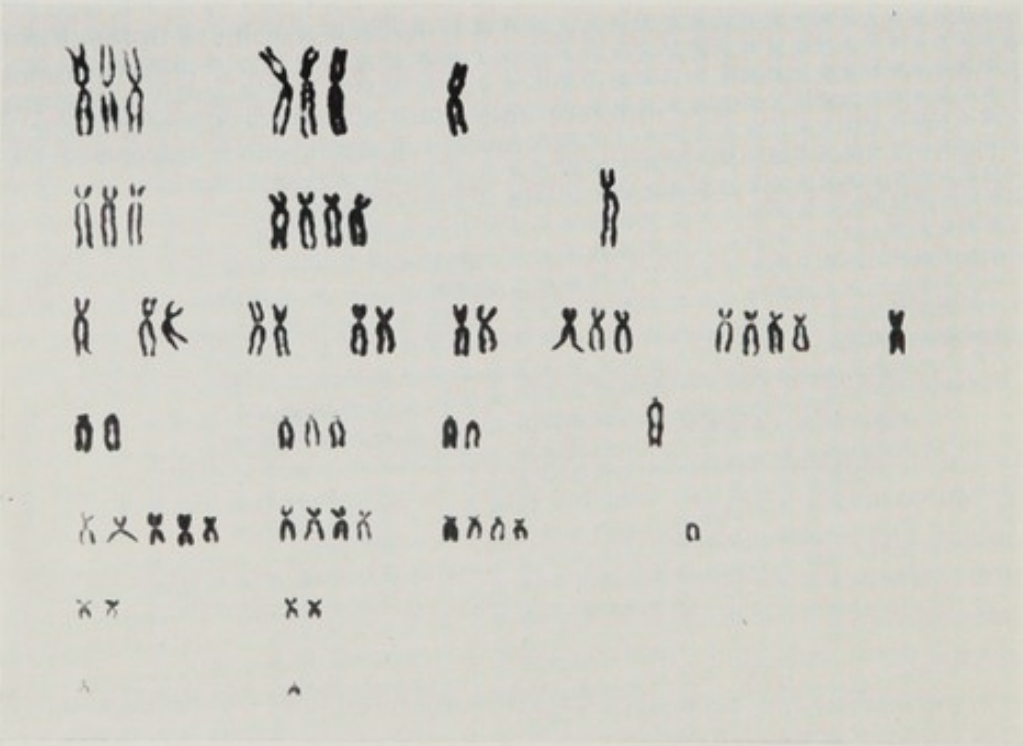


FIG. 15.2. Karyotype of the Sub.1 cell line (de Carli *et al.*, 1963).

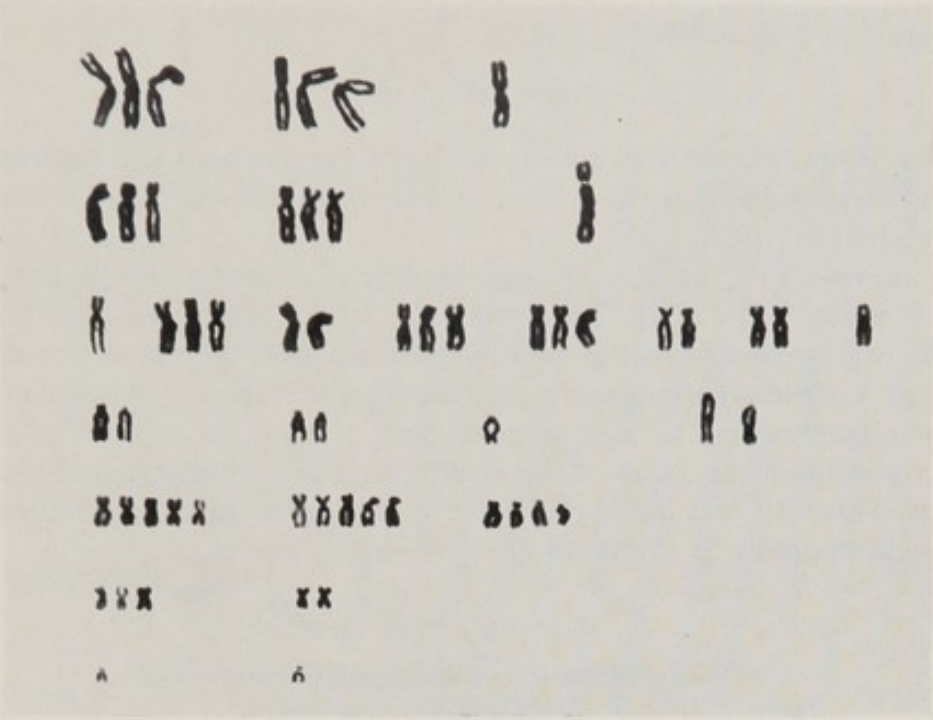


FIG. 15.3. Karyotype of the Sub.2 cell line (de Carli *et al.*, 1963).

very high activity is seen for the EUE† heteroploid strain of epithelial morphology. Stable clones were isolated from this parent strain and some of them showed a sudden fall in enzyme activity (Maio and de Carli, 1962). Chromosome analysis of the initial EUE strain and the derived lines Sub-1 and Sub-2 (Figs. 15.1, 15.2 and 15.3) was undertaken together with estimation of alkaline phosphatase activity (Table 15.2). It is clear that the most noteworthy karyotypic difference consisted of diminution of the small acrocentric chromosomes 21 or 22 in the Sub-1 and Sub-2 lines which became comparable with normal cells for this group of chromosomes.

The enzyme activity of these cell lines was very low and comparable with that of the primary cultures. However, the very raised enzyme activity of the EUE strain cannot be explained simply by an increase in the number of small acrocentric chromosomes. It is reasonable to believe that this discordance is related to other chromosomal rearrangements observed. While it is true that chromosome 21 acts in one way or another on the level of alkaline phosphatase activity (direct control or regulation) it is not the only chromosome implicated.

B. Trisomy 21 and galactose-1-phosphate uirdyl transferase

Galactose-1-phosphate is metabolized in the young child by a reaction with uridine diphosphoglucose (UDPG) and this stage which leads to uridine diphos-

† Epithelio umano embrionale (human embryonic epithelium).

phogalactose is catalysed by galactose-1-phosphate uridyl transferase (Kalckar, Braganca and Monch-Petersen, 1953).

$$\text{Gal-1-P} + \text{UDPG} \rightleftharpoons \text{UDP gal} + \text{G-1-P}$$

This enzyme is inactive in galactosaemia of the newborn and heterozygotes for the unfavourable gene show about 50 per cent of the activity of normal subjects (Hsia *et al.*, 1960).

The activity of this enzyme has been studied in oligophrenics (Brandt, 1962, 1963; Brandt *et al.*, 1963b). The mean value of the results obtained for these subjects is very close to that in normal controls. However, the distributions of the results are not superimposable and it appears that there is bimodal distribution in oligophrenics. In fact, patients with trisomy 21 show high enzyme activity and the mean value of the results is 1·4 times higher than for normal subjects. This ratio was here again close to that of the genic material equal to 1·5 for chromosome 21 (Table 15.3).

TABLE 15.3

Activity of galactose-1-phosphate-uridyl-transferase (μ mole of UDPG/hr/g of haemoglobin) in normal, oligophrenic non-trisomic 21 and trisomic 21 subjects (after Brandt et al., *1963)*

Type of subject	Normal	Oliophrenic	Trisomic 21
Number of subjects	452	142	27
Activity	26·4	27·0	36·9
Standard deviation	3·7	3·8	5·16

The assays were carried out on haemolysates of total blood and it seems reasonable to suppose that the nucleated precursors of erythrocytes form the enzyme. It may also be supposed that the plasma constituents have no effect on the assay of galactose-1-phosphate-uridyl transferase. The haemoglobin level and number of leukocytes and reticulocytes were within normal limits for all the blood samples.

C. Trisomy 21 and tryptophan metabolism

Study of biochemical disturbances in human subjects with karyotypic anomalies may be based on observation of anomalies in the formation of certain metabolites in relation to the normal metabolic pattern in healthy subjects. This stage thus precedes direct estimation of the enzyme activity in optimal conditions and in appropriate tissues. Such a situation is frequent. For example, the general metabolic anomaly of galactosaemia of the newborn and even its therapy were known before it was possible to pinpoint the lack of activity of galactose-1-phosphate-uridyl transferase in these subjects.

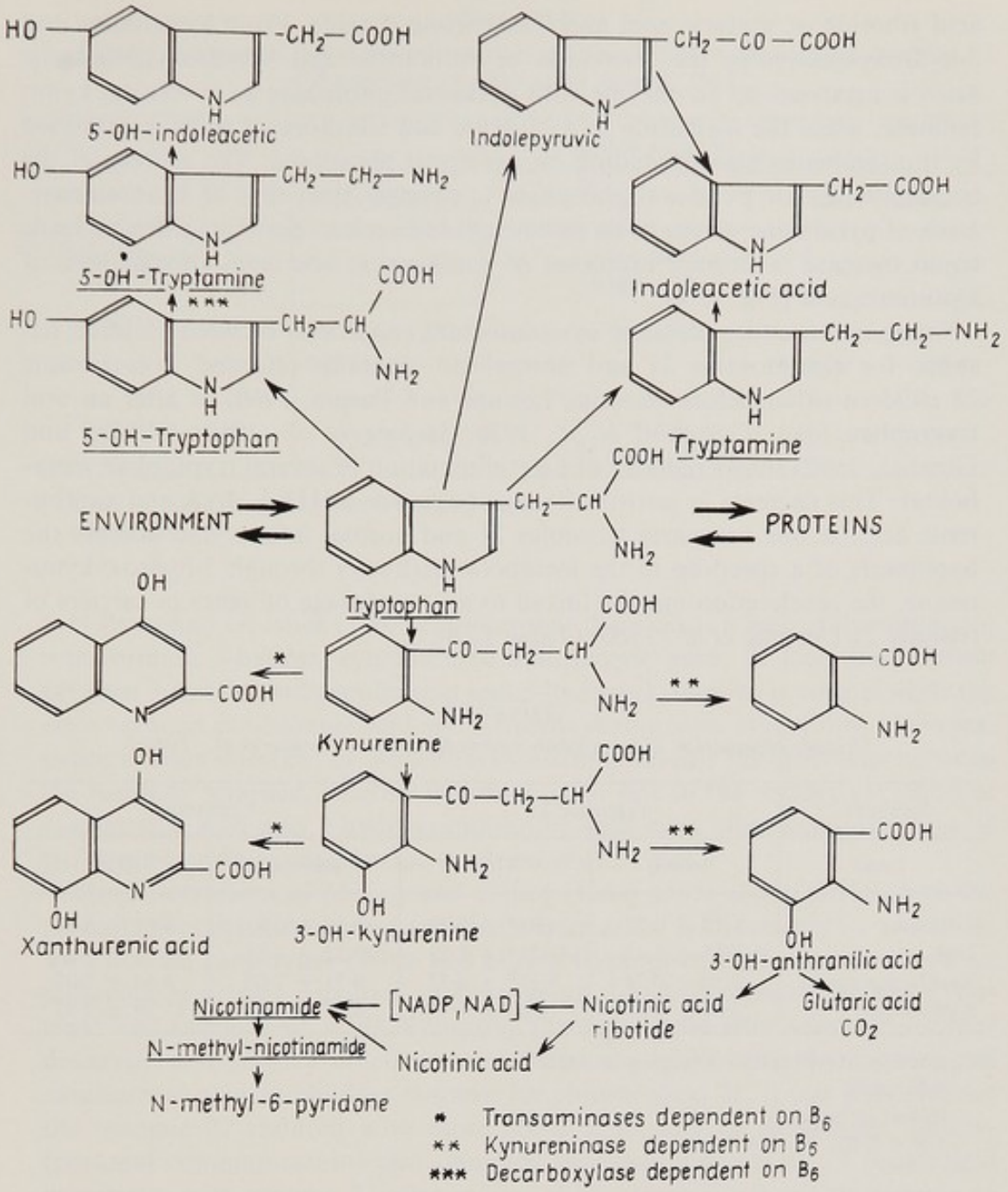


FIG. 15.4. Scheme of tryptophan metabolism.

The results of investigation of tryptophan metabolism in children with trisomy 21 may correspond to this possibility.

In the human body 1–3 per cent of exogenous tryptophan is transformed either into serotonin and 5-hydroxyindoleacetic acid (5-HIAA), on the one hand, or into indoleacetic acid (IAA) on the other. The bulk of the tryptophan is catabolized by opening of the indole ring giving kynurenine, then by oxidation, for which the enzyme cofactor is NADP, giving 3-hydroxykynurenine. Normally, small quantities of anthranilic, kynurenic and xanthurenic acids form. The bulk of the 3-hydroxykynurenine is catabolized in its turn, giving either nicotinic

acid ribotide or glutaric acid and then carbon dioxide. From kynurenine and 3-hydroxykynurenine the formation of anthranilic and 3-hydroxyanthranilic acids is catalysed by an enzyme with pyridoxal phosphate as co-factor, kynureninase, while the formation of kynurenic and xanthurenic acids is catalysed by transaminases also depending on pyridoxal phosphate. The affinity of the transaminases for pyridoxal phosphate is stronger than that of kynureninase. Lack of pyridoxine or use of an antimetabolite such as desoxypyridoxine leads to an increase in urinary excretion of xanthurenic acid and, though less, of kynurenic acid (Fig. 15.4).

Comparison under identical environmental conditions between children trisomic for chromosome 21 and normal or mentally retarded non-trisomic 21 children either before (Jerome, Lejeune and Turpin, 1960) or after an oral tryptophan load (Gerschoff *et al.*, 1958; Jerome *et al.*, 1960; O'Brien and Groshek, 1962) shows reduced urinary elimination of several tryptophan metabolites. This decrease is statistically significant for 5-HIAA, IAA and xanthurenic acid. If one compares trisomics 21 and normal infants and accepts the hypothesis of a speed-up in the metabolic pathways through 3-hydroxykynurenine, the acceleration may be linked to an overdosage of genes in carriers of trisomy 21 (Jerome *et al.*, 1960) (Table 15.4).

TABLE 15.4

Urinary elimination of tryptophan metabolites (after Jerome et al., *1960)*

Subjects	Trisomic 21		Controls	
Load	Before	After	Before	After
5 HIAA	1·75 ± 0·22	2·68** ± 0·19	3·33 ± 0·41	4·57 ± 0·58
IAA	9·00** ± 1·42	14·42** ± 2·29	20·32 ± 4·58	31·45 ± 4·65
Xanthurenic acid	2·30 ± 0·74	5·31* ± 0·52	4·71 ± 1·00	8·64 ± 1·07
Kynurenine	2·05 ± 0·19	8·63 ± 1·75	2·17 ± 0·45	11·29 ± 2·52

Load = l-tryptophan 30 mg/kg by mouth.
8-hr urine.
Result in μg/kg/hr.
* $P < 0{\cdot}05$, ** $P < 0{\cdot}01$.

It may be shown that the reduced formation of 5-HIAA is not due to defective decarboxylation of 5-hydroxytryptophan. In fact, subcutaneous injection of 5-hydroxytryptophan leads to considerable increase in the formation of 5-HIAA which becomes similar both in subjects with trisomy 21 and the controls (Jerome, 1962) (Table 15.5).

Reduced renal elimination of xanthurenic acid has been noted by several authors when comparing mental defectives—children with trisomy 21 or with normal karyotypes after a tryptophan load (Gershoff *et al.*, 1958; O'Brien and Groshek, 1962) or trisomics 21 with normal children before and after a tryptophan load (Jerome, 1960).

TABLE 15.5

Urinary excretion of 5-hydroxyindolacetic acid before and after subcutaneous injection of 5-hydroxytryptophan (after Jerome, 1962)

	5 HIAA μg/kg/hr		
	Before treatment		After treatment
Trisomics 21 No = 6	3·63 ± 0·42	t = 8·7 0·001 > P	13·23 ± 1·03
	t = 3·6 0·01 > P > 0·001		t = 0·47 0·70 > P > 0·60
Controls No = 6	6·37 ± 0·64	t = 3·2 0·01 > P > 0·001	14·47 ± 2·44

Load = dl-5-OH-tryptophan 0·5 mg/kg subcut.
8-hr urine.

O'Brien and Groshek (1962) consider that there exists a lack of transaminase transforming 3-hydroxykyurenine to xanthurenic acid. In fact, the relation between 3-hydroxyanthranilic acid and 3-hydroxykyurenine is normal while the ratio between xanthurenic acid and 3-hydroxykynurenine is very low. However, subcutaneous injection of 3-hydroxykynurenine reveals no difference between the mentally retarded, whether trisomics 21 or not, in the urinary excretion of xanthurenic acid and 3-hydroxyanthranilic acid even after administration of pyridoxine or desoxypyridoxine (O'Brien *et al.*, 1962).

The observations of McCoy and Chung (1964) are in contradiction with the preceding results. Comparing the mentally retarded with trisomy 21 and those with normal karyotypes before and after a tryptophan load, they observed no difference in urinary excretion of kynurenine, 3-hydroxykynurenine and kynurenic and xanthurenic acids. Repeating the experiment after administration of desoxypyridoxine they noted heavier elimination of 3-hydroxykynurenine and xanthurenic acid in children trisomic for chromosome 21. It was possible that the trisomic 21 children were more sensitive to lack of pyridoxine. McCoy (personal communication) also observed increased elimination of oxalic acid in patients with trisomy 21 after intake of desoxypyridoxine. This appears to confirm sensitivity to lack of pyridoxine in these subjects and to be in agreement with the observations of Gershoff *et al.* (1959) showing a greater transformation of pyridoxine into 4-pyridoxic acid in trisomy 21. However, these authors (Gershoff *et al.*, 1958) observed a diminution in the formation of xanthurenic acid in trisomy 21 after a tryptophan load. In addition, if it is a question of sensitivity to the competitive effect of desoxypyridoxine more marked in trisomy 21 than in controls it is surprising that no effect on the formation of kynurenic acid was observed. If it is a question of lack of affinity of kynureninase for its co-factor we must assume that there exist two distinct kynureninases specific for 3-hydroxykynurenine and kynurenine.

It is difficult in these trials to assess the importance of tryptophan metabolism by the action of the intestinal bacterial flora. It may be that the reduction in urinary elimination of certain tryptophan metabolites may also be explained by reduced or retarded intestinal absorption. In order to eliminate the possible changes in intestinal absorption it is possible to inject intravenously a solution of this amino acid in a fairly concentrated form in an attempt to saturate the enzyme systems (Jerome, 1964). The 5-HIAA and the different metabolites of the principal pathway via kynurenine are related to the sum of the tryptophan and the derivatives of tryptophan eliminated during the same period. All the terms of the ratio correspond to quantities of metabolites eliminated after a load in excess of the amounts eliminated during the same period before the load. Under these conditions we still observe a reduction in the formation of 5-HIAA but, on the other hand, an increase in the derivatives estimated in the main metabolic pathway (Table 15.6).

TABLE 15.6

Urinary elimination of 5-hydroxyindolacetic acid and of the sum of several derivatives of formyl-kynurenine in trisomic 21 and non-trisomic 21 mental defectives (after Jerome, 1964)

	No. tris. 21 = 8	No. controls = 8
5 HIAA × 10	0·126* ± 0·013	0·185 ± 0·017
Glob. Ind. + Kyn. + OH-kyn. + OH-anthra + NMPC		
Kyn. + OH-kyn. + OH-anthra + NMPC	0·511** ± 0·053	0·308 ± 0·040
Glob. Ind. + Kyn. + OH-kyn. + OH-anthra + NMPC		

* $P < 0{\cdot}05$, ** $P < 0{\cdot}01$.

Glob. Ind. = global indole derivatives (including tryptophan).
Kyn. = kynurenine.
OH-kyn. = 3-hydroxykynurenine.
OH-anthra = 3-hydroxyanthranilic acid.
NMPC = N-methylpyridone carboxamide.
5 HIAA = 5-hydroxyindoleacetic acid.
Load = l-tryptophan 50 mg/kg intravenously.
8 hr-urine.

According to these results it is possible that in children with trisomy 21 there is acceleration of the metabolic pathway via kynurenine. One or more genes exercizing a structural role or control of the formation or functioning of the enzymes involved in the degradation of tryptophan may be situated in chromosome 21. The exact position of this anomaly in the metabolic chain cannot be definitely stated at the present time. It can only be confirmed by study of enzyme activity of tissue preparations.

D. Variance of the experimental results with genic overdosage

All the results reported of the determinations whether of enzyme activity or elimination of metabolites show that there exists a ratio of the variance to the mean V/M greater in trisomics 21 than in controls whatever the precautions taken. According to a theoretical model proposed by Lejeune, if two alleles of frequency p and q are considered giving additive quantitative effects a and b, it in may be expected that V/M will be identical in a normal subject and a trisomic due to meiotic non-disjunction, while for the same series of observations V/M will be 1·67 times greater in a trisomic through lack of mitotic segregation.

TABLE 15.7

Value of V/M for different investigations comparing trisomic 21 subjects and controls

	Trisomics 21			Controls			V/M controls
	M	V	V/M	M	V	V/M	V/M tris.
Trubowitz *et al.*, 1962	60	729	12·15	40	289	7·23	1·68
Trubowitz *et al.*, 1962	70	1444	20·63	40	225	5·63	3·66
Lennox *et al.*, 1962	69	256	3·71	48	144	3·00	1·24
King *et al.*, 1962	112	1681	15·01	92	1296	14·09	1·07
King *et al.*, 1962	120	1681	14·01	91	1764	19·38	0·72
Brandt *et al.*, 1963	36·9	26·6	0·72	26·4	13·7	0·52	1·38
Jerome (kynurenine and derivatives)	0·511	0·234	0·46	0·308	0·101	0·33	1·39
V = Variance (σ^2), M = Mean						General average	1·59

The experimental values tabulated in Table 15.7 show that the mean of the ratio of the different values for V/M is equal to 1·59 which appears to show that trisomy 21 appears more often through lack of mitotic segregation, which would agree with cytogenetic arguments (Lejeune, 1963).

SEX CHROMOSOME LINKED BIOCHEMICAL EFFECTS

The appearance of an unfavourable mutation for a gene directing synthesis of an enzyme in a heterozygous subject brings about a 50 per cent reduction in enzymatic activity. Several examples are known such as acatalasaemia but these genes are always located on the autosomal chromosomes. For those found to be X chromosome-linked, this does not appear to be the case. Thus, the gene controlling synthesis of glucose-6-phosphate dehydrogenase (G-6-PD) is situated on the X chromosome and yet cell enzyme activity is identical in the XX female and the XY male. It seems that at interphase while the invisible chromosomes direct protein synthesis then double their DNA content, one of the X

chromosomes is inactive and appears in the form of sex chromatin or the Barr body (Ohno and Makino, 1961). A single X chromosome therefore appears to be active and this would extend to sex chromosome anomalies since we see in the normal XX female one Barr body, two bodies in an XXX syndrome and three in an XXXX syndrome. Inactivation may occur as early as the twelfth day of embryonic development and affect at random either the paternal X or maternal X chromosome. A double cellular population exists in the female, one with an active maternal X, the other with an active paternal X chromosome (Lyon, 1961).

A. Quantitative aspects of the inactivation of the X chromosome

The gene of G-6-PD deficiency is helpful in the study of the inactivation of the X chromosome. Many females heterozygous for the unfavourable gene show cell enzyme activity only half of that measured in a female homozygous for the normal gene. If the hypothesis is true, half the cells of these subjects carry an active X chromosome with a defective gene and their activity will be nil while the other half of the cells carry an active X with normal gene and their activity will be identical with that of all the cells of normal male or female subjects.

Considering this possibility, Beutler, Yeh and Fairbanks (1962) studied the G-6-PD activity of the erythrocytes, which are particularly accessible material, reasonably assuming that the nucleated precursors of these cells form the enzyme. Oxidation of glucose-6-phosphate being coupled with the maintenance of glutathione in the reduced state (GSH), a measure of the speed of disappear-

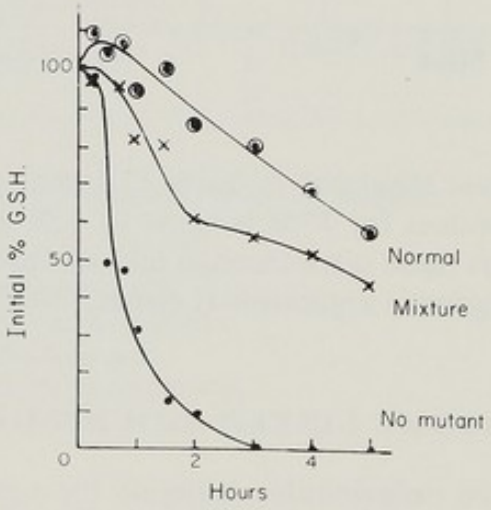


FIG. 15.5. Rate of disappearance of GSH from the blood of a male hemizygous for the gene of G-6-PD deficiency, a normal subject and in an artificial mixture of the two blood samples (Beutler *et al.*, 1962).

ance of GSH, may be used to differentiate two cell populations from the total blood. The level of GSH in normal blood falls slowly while it drops rapidly in the blood of a subject without G-6-PD such as a male hemizygous for the unfavourable gene. The mixture of these two bloods gives a graph with a marked

inflexion at the time of oxidation of GSH of the deficient cells (Fig. 15.5). Using the blood of three heterozygous women the test gave curves comparable with those obtained with the mixture of normal blood and totally deficient blood (Fig. 15.6).

More recently, Beutler and Baluda (1964) separated two erythrocyte populations in females doubly heterozygous for the gene of haemoglobin S in sickle

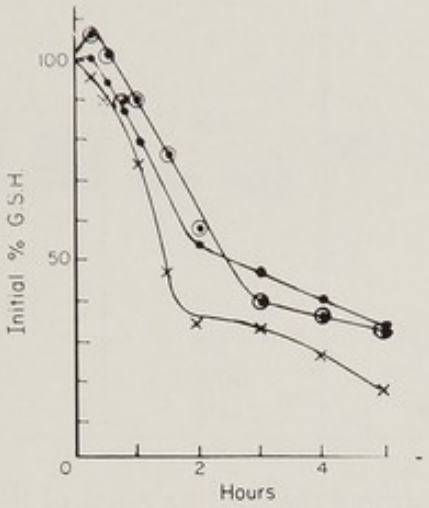


FIG. 15.6. Rate of disappearance of GSH from the blood of three females with "intermediate" G-6-PD blood activity (Beutler *et al.*, 1962).

cell anaemia and deficiency of G-6-PD. Separation was achieved thanks to the absence of sickling in cells rich in methaemoglobin. Haemoglobin was first completely oxidized to methaemoglobin, methaemoglobin being reduced in proportion to the activity of glucose-6-phosphate dehydrogenase of the erythrocytes. The cell suspension was then filtered in the absence of oxygen through a Millipore membrane. The cells rich in methaemoglobin and not sickled passed the membrane while most of the cells deficient in methaemoglobin and sickled were held back. Finally, glucose-6-phosphate dehydrogenase activity was estimated in the two types of cells: in the female heterozygous for deficiency of G-6-PD, the erythrocytes passing the filter are much poorer in G-6-PD than those held back, while in normal subjects, male or female, the levels of G-6-PD are identical.

An elegant demonstration might be made by preparing clones from a tissue culture of a female heterozygous for deficiency in G-6-PD. In this case we should obtain clones without enzyme activity alongside those with normal activity. This second possibility was recently explored (Davidson, Nitowsky and Childs, 1963). After showing that the enzyme activities of cutaneous cell cultures correspond to different possible genotypes (Table 15.8) these authors separated the clones from these primary cultures. Only the tissue cultures obtained for females heterozygous for the unfavourable gene gave a mixture of clones with almost no activity and with normal activity (Table 15.8).

It is a little disconcerting to note that a high proportion of women heterozygous for the mutant gene have in their blood almost zero G-6-PD activity or, on the contrary, almost normal activity. This variable reflection may depend

TABLE 15.8

Activity of G-6-PD per mg protein of skin biospy cells before and after cloning for various phenotypes in subjects of Sardinian origin (after R.G. Davidson et al.*)*

Subjects	Initial activity	Number of clones	Clonal activity
♀ Heterozygous female	6·3	1	12·9
		5	9·0
		3	8·7
		2	1·2
♀ Heterozygous female	2·4	16	10·3
		2	9·7
		15	9·1
		13	2·7
♂ Mutant hemizygous male	1·8	5	2·1
		2	1·2
		1	1·3
♀ Mutant hemizygous female	2·3	4	1·6
♂ Normal hemizygous male	10·4	1	13·7
♀ Normal hemizygous female	11·1	4	11·3

on the number of cells forming the starting point of the development of a particular tissue at the moment of inactivation of the X chromosome. If only two cells are involved, theoretically we may observe in a quarter of the heterozygous females studied, enzyme activity equal to that of females homozygous for the normal gene, in a quarter of these subjects no activity and in half of these subjects activity equal to half the activity of females homozygous for the normal gene. If the number of cells in question increases, the phenomenon would still be governed by a binomial distribution but the proportion of extreme activities would fall in favour of intermediate activities.

B. Qualitative aspects of inactivation of the chromosome

Inactivation of an X chromosome may explain the presence of a phenotypic mosaic expression in the female mouse heterozygous for an X chromosome-linked recessive gene affecting the skin colour (Lyon, 1961).

TABLE 15.9

Results of starch gel electrophoresis of enzymatic extracts of clones from females with electrophoretic AB phenotype for G-6-PD (after R.G. Davidson et al., *1963)*

Subjects ♀	1	2	3	4	5	6	Overall total
Clones Type A	8	1	0	8	7	0	24
Clones Type B	2	8	8	0	7	5	30
Clones Type AB	0	0	0	0	0	0	0
Individual total	10	9	8	8	14	5	

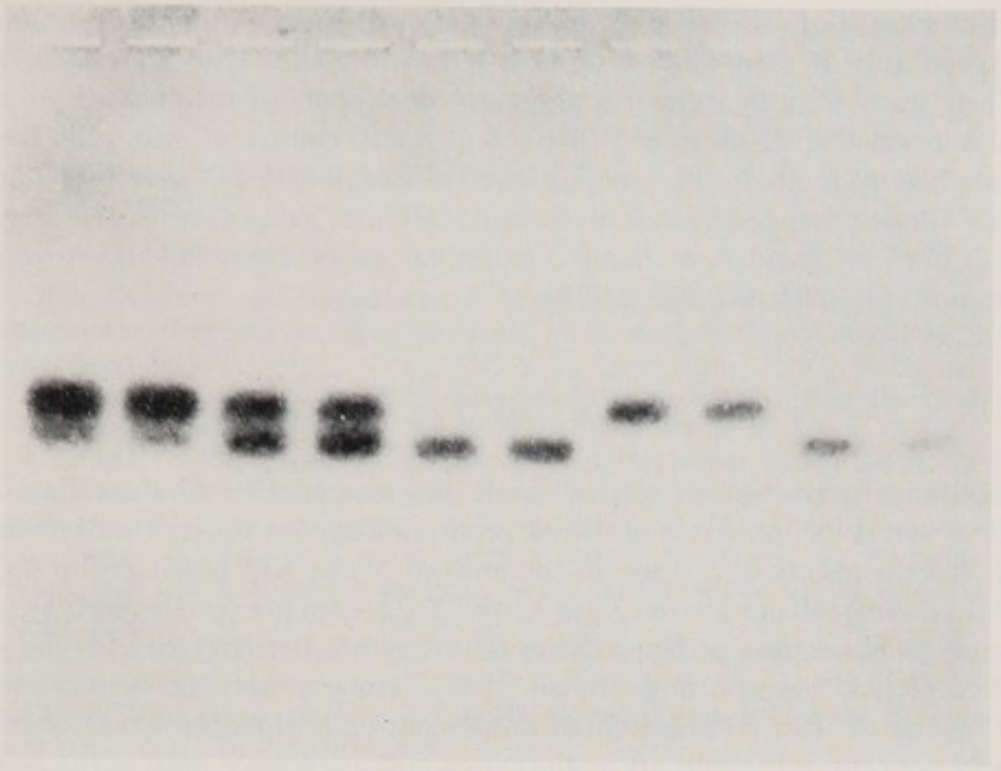


FIG. 15.7. Starch gel electrophoresis of G-6-PD from cultures of skin cells. The samples were run in duplicate and show from left to right two heterozygous AB phenotype females and three subjects with a single band 2 A and 1B (Davidson *et al.*, 1963).

FIG. 15.8. Starch gel electrophoretogram of enzymatic extracts of G-6-PD from cultured cells of an heterozygous female with AB phenotype. From left to right, extract of the original cell line and of nine derived clones (Davidson *et al.*, 1963).

In humans, G-6-PD exists in the form of two genetic variants separable by electrophoresis in starch gel: a rapid type A and a slow type B (Fig. 15.7).

Their study affords a decisive argument in support of inactivation of an X chromosome (Davidson *et al.*, 1963). A primary culture of skin cells from a female heterozygous for the qualitative character of G-6-PD gave two A and B bands. Clones obtained by unicellular reinoculations never gave the two bands A and B but only band A or band B in similar proportions for all six subjects examined (Fig. 15.8 and Table 15.9).

C. Partial inactivation of the X chromosome

Random inactivation of one of the X chromosomes in all the cells of the embryo seems incontestable but work reported does not suggest total inactivation. Recent results of a study of the antigen of the Xg^a blood group do not reveal a double cell population Xg(a+) and Xg(a−) in five females with Xg(a+) phenotype but whose pedigree shows that they are heterozygous for the gene studied (Reed, Simpson and Chown, 1963). Using an anti-Xg^a serum washed and incubated with an anti-human fluorescent goat globulin it was observed that 99 per cent of the cells are agglutinated and fluorescent both in the heterozygous Xg(a+) female and in the Xg(a+) male. If the Xg^a antigen in an erythrocyte does not come from another cell but more probably from a precursor of the erythrocyte, it must be supposed that inactivation of the X chromosome is partial and confined to certain regions or loci.

Labelled autosomal fragments have been translocated onto chromosome X of female mice (Russell, 1963). Under these conditions, we find that inactivation does not extend to all the attached fragment. There exists a gradient of inactivation over a limited distance.

It is possible that only the long arm of the chromosome X is inactivated. One may observe during interphase that the sex chromatin is extended as a fine thread corresponding to the still active part of the chromosome and perhaps to the short arm of the X chromosome (Reitalu, 1957).

Future perspectives

Study of relations between chromosomes and enzymes is of theoretical interest in mapping the genes of the chromosomes. It may also be of practical interest. While it is true that it is impossible to correct the chromosome anomaly itself, as it must often correspond to overdosage of genes, it is easier to imagine blocking of an enzyme, the excess of which leads to metabolic disequilibrium, than activation of a deficient enzyme. This might apply to incidents such as trisomy 21 in which the malformations are detectable at birth. In fact, we find in these subjects deterioration of mental functions with fall of the intelligence quotient which very clearly diminishes with time (Chapter 4). Therapy might be attempted but any palliative action implies knowledge of the enzymatic consequences of the chromosomal anomaly.

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Index

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